Investigation of Anti-Inflammatory Potentials of Terminalia Tomentosa Wight & Arn. Bark - An In-Vitro Approach

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Abstract

Terminalia tomentosa bark. Belongs to the family Combretaceae. Bark is astringent and useful in the treatment of ulcers, vata, fractures, hemorrhages, bronchitis and diarrhea. This study establish the anti-inflammatory activity of ethanolic (TEA) and aqueous (TEW) of T. tormentors aback extracts in in-vitro methods by Inhibition protein denaturation (BSA), Inhibition of Egg Albumin denaturation and The human HRBC membrane stabilization method. Alcoholic and aqueous extracts exhibited significant protein denaturation inhibition in egg albumin assay with IC₅₀ values TEA 113µg/mL, TEW 112.67µg/mL in comparison with Standard (Diclofenac sodium) 236.7µg/mL. In bovine serum albumin denaturation assay with IC₅₀ values TEA 16.91µg/mL and TEW 17.67µg/mL respectively when compared with standard (Diclofenac sodium) 67.21µg/mL. Both alcoholic and aqueous extracts were shown to have membrane stabilization properties with the IC₅₀ values TEA 92.95µg/mL, TEW 37.68µg/mL respectively, in comparison with standard (Diclofenac sodium) 47.48µg/mL. To conclude, this study shows that the T. tormentors, possess significant anti-inflammatory activity.

Keywords: Terminalia tomentosa, Anti-inflammatory, Combretaceae, HRBC, Egg albumin Protein denaturation.

Introduction

Nature’s fauna and flora gave us a complete store of remedies to treat the ailments of suffering mankind, hence these natural drugs have been used since ages as cure for diseases as they contain natural chemical compounds of therapeutic value of drugs[1].

Phytochemicals present in fruits, vegetables, natural plants are the main sources natural sources for treating various ailments[2]. A number of modern drugs have been obtained from the natural sources, many research protocols have discussed the need of medicinal plants as sources of new curative therapeutic analogs[3]. All the organisms require the elimination of foreign bodies like pathogens, and injured tissues and whose functions are mediated by a complex host response known as inflammation. It is a defensive response intended to exclude the cause of cell injury. Acute inflammation is a speedy response to injuries or microbes and other external substances, which is designed to deliver plasma proteins and leukocytes and to sites of injury. Chronic inflammation is a prolonged duration of inflammation in which active inflammation, healing and tissue injuries, proceed simultaneously[4].

Chronic inflammation leads to several pathological conditions e.g. atherosclerosis, arthritis, hypersensitivity as well as cancer and other various autoimmune diseases. For most of these conditions no satisfying treatments are available. Common treatment is given by use of steroidal and NSAIDs which having higher adverse side effects[5]. Due to the more side effects of
steroidal and NSAIDs drugs, there is an increasing interest in herbal compounds, such as herbal remedies and dietary supplement which have been used for ages as a traditional treatment to reduce pain and inflammation.[6]

Plant Terminaliatomentosa Wightman, belongs to the family Combretaceae, also known as crocodile bark known to contain carbohydrates, steroids, flavonoids, triterpenoids, tannins and spooning. The plant has been known to possess various pharmacological activities like anti-leucorrhoea, anti-hyperglycemic, antioxidant, antifungal, anti-diarrheal etc. The bark of the plant is astringent and useful in bronchitis, ulcers, hemorrhages, vata, fractures, diarrhea etc.

Photochemical investigation of T.tomentosa bark confirms the presence of flavonoids, polyphenols and tannins and the bark is not investigated for its anti-inflammatory activity.

Materials and Methods

T. tomentosabark was collected from Somas hare forest Hebri, Manipal, in September 2014, authenticated by Dr. Gopala Krishna Bhat, Taxonomist, Taxonomy Research Centre, PP College Udupi. A herbarium specimen (PP 600) was kept in the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal.

Morphology and Distribution

A large deciduous tree, thick bark, dark coloured and deeply fissured outside and light dark coloured inside. Leaves sub opposite or alternate, up to 17 x 6cm, elliptical or obovate- oblong, obtuse or acute at apex, narrowed at base, glands stalked, some way up the midrib beneath. Flowers are yellowish, in erect terminal and axillary panicles. Fruits are 4.5-5cm long with usually 5 wings. This tree is fairly common in forests.

Throughout India, very commonly found in the sub-Himalayan tracts, deciduous forests of Nepal, Sikkim, ascending to 1300m, except arid regions like Punjab and Rajasthan. [7]

Extract Preparation

Bark was dried in an air circulation oven at 37 °C, and coarsely powdered. The aqueous extract was prepared by macerating the powder with chloroform and water in the ratio of 2:98 for 7 days with occasional shaking, extract was filtered, evaporated. Ethanolic extract was prepared by subjecting the plant material to soxhlet using the solvent ethanol. Concentrated by distillation. The percentage yields were calculated for both the extracts and were stored at 4-5°C in a refrigerator.

In-Vitro Anti-Inflammatory Studies

Inhibition of Bovine Serum Albumin Denaturation (BSA)

The method as described earlier [8] was adopted with minor modifications. In brief the reagent mixture (0.5 mL; pH 6.3) contain 0.45 mL of BSA (5% aqueous solution) and 0.05 mL of various concentrations of test or standard drug was added to the reaction mixture and for control samples 0.05 mL of double-distilled water was added replacing the test compound. Then it was incubated for 30 min at 37 ± 2°C , heated at 70 ± 2°C for 30 min followed by cooling. After cooling the samples 2.5 mL of phosphate buffer solution was added and mixed slowly.

The resulting turbidity was measured spectrophotometrically at 600 nm (SHIMADZU, UV 1605 PC) and the % protein denaturation inhibition was calculated.

\[ % \text{ inhibition} = 100 \cdot \left( \frac{V_t}{V_c} - 1 \right) \]

Where, \( V_t = \) absorbance of test sample, \( V_c = \) absorbance of control.

Egg Albumin Denaturation Inhibition

The method was described earlier [9] was followed with minor modifications. The reagent mixture (5 mL) contains of 0.2 mL of egg albumin from hen’s egg, 2.8 mL of phosphate buffered saline (PBS pH 6.4) and 2 mL of test and standard drugs of varying concentrations and for control samples 0.05 mL of double-distilled water was added in
place of test compounds. Then the solutions were mixed slowly by shaking the test tubes. The mixtures were incubated at 37± 2°C for 15 min and then it was kept in water bath at 70± 2°C for 5 min. mixture was cooled and the absorbance taken at 660nm, using vehicle as blank. The % protein denaturation inhibition was estimated by considering denaturation in control as 100 percent and was calculated by using the formula:

\[
% \text{ inhibition} = 100 \times \left(\frac{A_t}{A_c} - 1\right)
\]

\*A_t = absorbance of test/ standard sample, A_c = absorbance of control sample.

**The Human Red Blood Cell (HRBC) Membrane Stabilization Method**

Blood obtained from healthy human volunteer who had not taken any NSAIDs for two weeks and was mixed with equal volume of sterilized Alsever, followed by centrifugation at 3000 rpm for 10 minutes, packed cells were separated and were washed in normal saline solution and a 10% v/v suspension was made with normal saline. The assay mixture consists of various concentrations of plant extracts, standard drug (Diclofenac sodium) were mixed with phosphate buffer 1mL separately, 2 mL of hypo saline and 0.5 mL of human RBC suspension. For control sample normal saline was added instead of test compound. Incubated the assay mixtures at 37°C for 30 min followed by centrifugation at 3000 rpm for 10 minutes. Separated the Supernatant liquid and the content of hemoglobin was estimated by a spectrophotometer at 560 nm.¹⁰

The % haemolyses was estimated by considering haemolysis in the control as 100%.

\[
% \text{ Protection} = 100 - \left(\frac{\text{Absorbance of test/Absorbance of control}}{1} \times 100\right)
\]

**Results**

**BSA Denaturation & Egg Albumin Denaturation inhibition Assay**

Effects of aqueous extracts and ethanol extracts of T. tomentosa on inhibition of heat-induced denaturation of Bovine Serum Albumin (BSA) and egg albumin were compared with that of standard NSAID i.e., diclofenac sodium. In our experimental study it was observed that ethanolic and aqueous extract of T. tormentors bark demonstrated significant activity.
Discussion

Inflammation constitutes of highly correlative set of events which allows the organs to respond against stimulus. The mechanism of action of NSAIDs is still obscure, but most of the NSAIDs interact with plasma proteins and many acidic NSAIDs balance serum albumin and also other fractions against heat coagulation. At lower concentration NSAIDs do not change the protein conformation directly and also do not inhibit the specific protein combination, but they do impact the conformational changes affected by some proteins on heating. This suggests that NSAIDs interact in some or the other way with proteins. Based on this property it was thought that anti-inflammatory drugs may have some connection its activity with inhibition of protein denaturation.[11]

This study demonstrated that the ethanolic and aqueous extracts of T. tomentosa possess significant property of protein denaturation protection and it was comparable to diclofenac sodium.

Both aqueous and alcoholic extracts of T. tormentors had significant membrane stabilization property. RBC membrane is identical to the membrane of lysosome and its maintain by plant extracts implies secure of this membrane is a major step considered in reducing the response to inflammation, by preventing the release of its constituents of activated neutrophil, such as proteases and bactericidal enzymes, which leads to further damage to the tissue.[10]

Conclusion

To conclude, T.tomentosapos sesses significant anti-inflammatory potential. Future studies are necessary to provide deeper insight into the mechanism of the action of anti-inflammatory activity by T. tomentosa bark.

Declaration of Interest

The authors report no declaration of interest.

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