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COMPARATIVE STUDY OF ANTI-ARTHRITIC ACTIVITY OF DIFFERENT EXTRACTS OF *THEVETIA PERUVIANA LEAF*

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ABSTRACT

The present study is aimed to evaluate the leaf extract of *Thevetia peruviana* for acclaimed anti-arthritic activity using *in-vitro* inhibition of protein denaturation model. Diclofenac sodium was used as a standard drug. Results revealed that the chloroform extract of *Thevetia peruviana* possessed significant anti arthritic activity as compared to aqueous and ethanolic extracts and standard drug diclofenainc sodium. The plant leaf extract showed dose dependent activity.

KEYWORDS: *Thevetia peruviana*, Anti-Arthritic, Diclofenac Sodium, Bovine Serum albumin.

INTRODUCTION

DISEASE INTRODUCTION

Rheumatoid arthritis is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage. Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells. It is a common disease having peak incidence in 3rd to 4th decades of life with 3-5 times higher preponderance in female. Its prevalence depends upon age.[4] The commonly used drug for management of inflammatory conditions are non-steroidal anti-

inflammatory drugs (NSAIDs), which have several adverse effects especially gastric irritation leading to formation of gastric ulcers. Herbal drugs constitute a major part in all the traditional system of medicine. Herbal medicine is a triumph of popular therapeutic diversity. The factors responsible for the continued and extensive use of herbal remedies in India are their effectiveness, easy availability, low cost, comparatively less toxic effects and shortage of practitioners of modern medicine in rural areas. Number of synthetic medicines has been derived from medicinal herbs. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

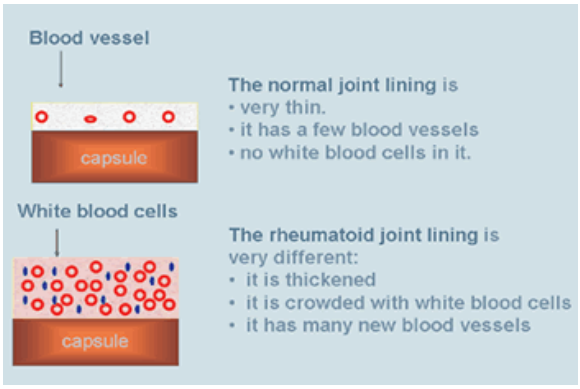

Fig. 1: Difference between normal joint lining and rheumatoid joint lining.

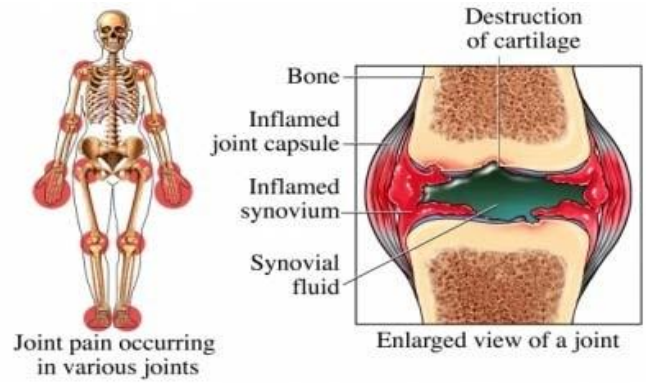

Fig. 2: Rheumatoid arthritis joint.

Thevetia peruviana is used medicinally in Philippine Islands, Guiana, Brazil and Gold Coast. *Thevetia peruviana* is used to treat various inflammatory and cardiovascular diseases, beside the antiviral and antifungal properties. Generally, *Thevetia peruviana* leaves are applied in cardiac disorder, fever, ringworms and measles treatment. Moreover, the use of *Thevetia peruviana* tree in folk medicine is well –known. However no report are available on the Antiarthritic, Anti Diabetic activities of the plant. Hence the present study was taken up to bring out the above features.

MATERIAL AND METHODS

Collection of plant

The leaves of the plant (Cascabela thevetia L., Apocyanaceae) were collected from surroundings of Miryalguda town, Nalgonda dist., and Hyderabad India in the month of February and was identified by Dr.Venkateswarlu, HOD, Pharmacognosy, G.Pulla Reddy College of Pharmacy, (Affiliated to Osmania University) Hyderabad.

Preparation of extracts

Ethanolic extract

100g coarse powder of dried leaves of Thevetia peruviana was packed and subjected to soxhlet extraction for continuous hot extraction with 95% ethanol. Then the extract was filtered and filtrate was concentrated under vacuum using rotary vaccum evaporator and percentage yield was calculated along with morphological evaluation, and fluorescence analysis of extracts under ultraviolet light in three different wavelengths.

Chloroform extract

100g coarse powder of dried leaves of Thevetia peruviana was packed and subjected to soxhlet extraction for continuous hot extraction with Chloroform. Then the extract was filtered and filtrate was concentrated under vaccum using rotary vaccum evaporator and percentage yield was calculated along with morphological evaluation, and fluorescence analysis of extracts under ultraviolet light in three different wavelengths.

Aqueous extract

100g coarse powder of dried leaves of Thevetia peruviana was packed and subjected to soxhlet extraction for continuous hot extraction with water. Then the extract was filtered and filtrate was concentrated under vaccum using rotary vaccum evaporator and percentage yield was calculated along with morphological evaluation, and fluorescence analysis of extracts under ultraviolet light in three different wavelengths.

Preliminary phytochemical screening

The ethanolic extract obtained was subjected to various chemical tests as per procedure mentioned in the standard reference books KR. Khandelwal, 2002[15]; kokate CK, 2008[14]; kokate CK, AL, 2006, Harborne[12] and Trease and Evans.[13]

Test for Alkaloids: To the test solution in 10 ml methanol, add 1 % (w/v)HCl and any of Mayor's reagents, Wagner's reagent or Dragendroff reagent (6 drops). A creamish or brownish red or orange precipitate indicates the presence of alkaloids.

Test for Anthraquinones: To the test solution add a benzene drop and ammonia drop, a pink colour indicates the presence of anthraquininones.

Test for Flavonoids: 5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2 SO_4. A yellow color in each extract indicated the presence of flavonoids. The yellow color disappeared on standing. Few drops of 1 % aluminum solution were added to portion of each filtrate. A yellow color indicates the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow color indicates opposite test for flavonoids. To the test solution in 10 ml of ethanol add conc. HCl– Mg ribbon developing a pink-tomato red color indicates the presence of flavonoids.

Test for Coumarins: To the test solution add a drop of sodium sulphate developing yellow colour indicates the presence of coumarins.

Test for Phenols: To the test solution add a drop of ferric chloride. Developing of intense colour develops which indicates the presence of phenols.

Test for Saponins: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth, which indicates the presence of saponins.

Test for Steroids (Liebermann-burchard Test): 2 ml of acetic anhydride was added to the test solution along with 2 ml of conc. H SO4. The colour changed from violet to blue or green in some samples. This indicates the presence of steroid.

Test for Terpenoids (Salkowski Test): 5 ml of each extract mixed with 2 ml of chloroform, and 3 ml concentrated H_2 SO_4 was carefully added to form a layer. A reddish brown colour of the interface was formed to show positive results for the presence of terpenoids.

Test for Tannins: About 0.5 g of the leaves was dried and powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue black colouration.

Test for Amino Acid and Protein (Ninhydrin Reaction): Take 2-3 ml of sample solution in a test tube. Add 3-4 drops of ninhydrin solution and heat. Appearance of purple or violet colour indicates the presence of protein.

Test for Carbohydrates (Benedicts Test): Add 1 ml of Benedict's reagent to test tube and heat the mixture to boiling in a water bath for 2 minutes. The formation of an orange red precipitate due to the formation of a copper (I) oxide indicates the presence of reducing sugars.

Fluorescence Analysis: The analysis of extract under daylight is unreliable due to lack of fluorescence. So it was evaluated under daylight and UV light.

Chemicals and drugs

Bovine serum albumin(SD FINE), sodium chloride (SD FINE), potassium chloride (SD FINE), disodium hydrogen phosphate (SD FINE), potassium dihydrogen phosphate (SD FINE), HCl (SD FINE), Diclofenac Sodium.

Preparation of reagents

5% Bovine serum albumin (BSA)

Dissolved 5 g of BSA in 100 ml of water.

Phosphate buffer saline pH 6.3

Dissolved 8 g of sodium chloride (NaCl), 0.2 g of potassium chloride (KCl), 1.44 g of disodium hydrogen phosphate (Na2HPO4), 0.24 g of potassium dihydrogen phosphate (KH2PO4) in 800 ml distilled water. The pH was adjusted to 6.3 using 1N HCl and make up the volume to 1000 ml with distilled water.

EXPERIMENAL MODEL[16]

Method

1. Test solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%W/V aqueous solution) and 0.05ml of test solution of various concentrations.

2. Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of distilled water.
3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution.
4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml of Diclofenac sodium of various concentrations.

Various concentrations (100, 250, 500 µg/ml) of test dugs and standard drug diclofenac sodium (100, 250, 500 µg/ml) were taken respectively. 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 keep the samples at 57°c for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm. The control represents 100% protein denaturation. The results were compared with Diclofenac sodium. All determinations were done in triplicate. The percentage inhibition of protein denaturation can be calculated as-

Percentage Inhibition = 100 – [{(optical density of test control – optical Density of product control)/optical density of test solution} × 100

RESULT AND DISCUSSION

Table 1: Preliminary phytochemical screening of leaf extracts (chloroform, Ethanol and aqueous) of *T. peruviana.*

S.No.	Phytoconstituents	Ethanolic extract	Chloroform Extract	Aqueous Extract
1	Alkaloids	+	-	-
2	Amino acids	-	-	-
3	Anthraquinones	+	-	+
4	Coumarins	-	-	-
5	Carbohydrates	-	-	+
6	Flavonoids	+	+	+
7	Phenolics	+	+	-
8	Proteins	-	-	-
9	Steroids	+	+	+
10	Saponins	+	+	+
11	Tannins	+	+	-
12	Terpinoids	-	-	-

Table No 2: In-vitro Anti-arthritic activity of leaf extracts (chloroform, Ethanol and aqueous) of *T. peruviana.*

Drug	Concentration (mg/ml)	Absorbance (560nm)	Percentage protection
Diclofenac Sodium	100µg	0.136	48.53
	250 µg	0.345	79.71
	500 µg	0.459	84.75
Test Control of Chloroform Extract		0.01	
Chloroform Extract	100µg	0.012	50.00
	250 µg	0.017	64.70
	500 µg	0.027	77.77
Test Control of Aqueous Extract		0.001	
Aqueous Extract	100µg	0.013	46.15
	250 µg	0.016	56.25
	500 µg	0.022	68.18
Test Control of Ethanolic extract		0.007	
Ethanolic extract	100µg	0.014	35.77
	250 µg	0.017	47.05
	500 µg	0.021	57.14

Results are expressed as Mean ±SEM (n = 6). Values are in minutes.

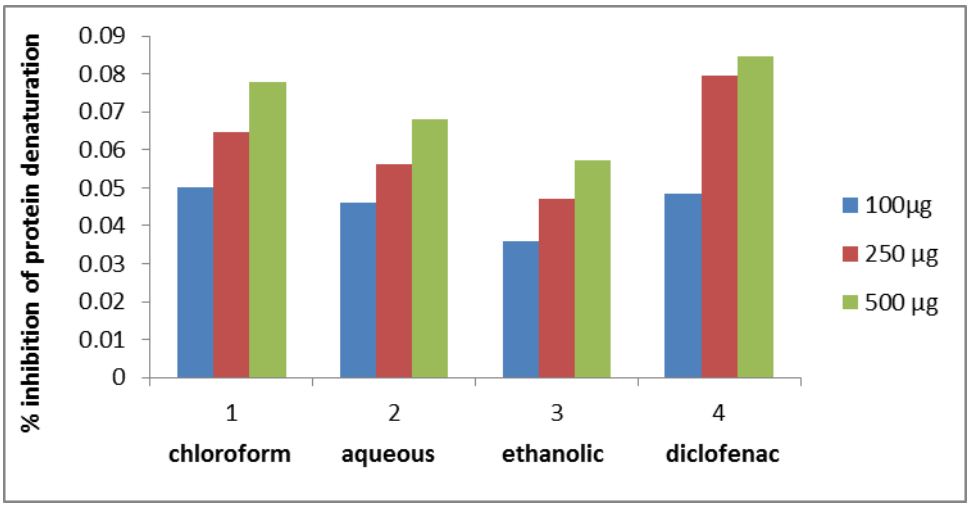

Fig 3: Percentage inhibition of protein denaturation.

DISCUSSION

Denaturation of protein is one of the cause of rheumatoid arthritis was well documented. Production of auto antigen in certain arthritic disease may due to denaturation of protein. The mechanism of denaturation probably involves alteration of electrostatic hydrogen, hydrophobic and disulphide bonding. From the result of the present study, it can be stated

that all the extracts of *T. peruviana* leaves is capable of controlling the production of auto antigen and thereby it inhibit the denaturation of proteins and its effect was compared with the standard drug diclofenac sodium. The percentage protection was found to be 77.77% (Chloroform), 68.18% (water) and 57.14% (ethanol) and 84.75% (Diclofenac sodium). All the extracts showed dose dependant response. This effect may be due to the presence of steroids, alkaloids, tannins, saponins and flavonoids present in various fractions. The effect was represented as follows

Chloroform extract > Aqueous extract > Ethanolic extract

CONCLUSION

The Invitro studies on leaves of *T. peruviana* showed the presence of significant anti- arthritic activity. The chloroform extract shows more anti-arthritic activity than water and ethanolic extracts. The Activity may be due to the presence of terpenoids, steroids, alkaloids, flavonoids and tannins. Our future aim is to isolate the chemical constituents responsible for the above activity and also to carry out the in vivo investigation.

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