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Evaluation of Anti-inflammatory activity of Ethanolic leaf extract of *Tephrosia villosa*

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A B S T R A C T

Tephrosia villosa belongs to the family Fabaceae. It is one of the most important plants used in the indigenous system of medicine. To study the anti-inflammatory activity of ethanolic leaf extract of *T.villosa* using Carrageenan induced model. Anti-inflammatory activity of leaf of *T.villosa* was studied, in which inflammation was induced by injecting *0.1ml of a freshly prepared solution of 1% carrageenan in 0.9% sodium chloride solution, subcutaneously.* Test drugs were administered in a dose of *200,400 mg/kg p.o.* one hour before commencing the experiment. The anti-inflammatory activity was assessed by determining and comparing the paw volume (ml) in the test drug group with that of the vehicle control group. *Diclofenac sodium* 25 mg/kg p.o. was used as a reference drug. Inflammation in the ethanolic leaf extract of *T.villosa* treated animals was found to be significantly less compared to vehicles control animals. (5 *Diclofenac sodium* (25mg/kg p.o.) produce significant reduction in inflammation when compared to control group. Ethanolic leaf extract of *T.villosa* produced significant anti-inflammatory activity. Our results suggest that all the leaf extract of *T.villosa* possess significant anti-inflammatory activity. Among the two doses of *T.villosa* 400 mg/kg p.o. showed maximum activity.

Keywords: *Tephrosia villosa*, Inflammation, Carrageenan

A R T I C L E I N F O

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1. Introduction

High-performance liquid chromatography (HPLC) is the In India, around 15000 medicinal plants have been recorded however traditional communities are using only 7,000 - 7,500 plants for curing different diseases. The medicinal plants are listed in various indigenous systems such as Siddha (600), Ayurveda (700), Amchi (600), Unani (700)

and Allopathy (30) plant species for different ailments. According to another estimate 17,000 species of medicinal plants have been recorded out of which, nearly 3,000 species are used in medicinal field. Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants. The valuable medicinal properties of different plants are due to presence of several constituents i.e. saponins, tannins, alkaloids, alkenyl phenols, glycol-alkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbolesters. Among them some are act as synergistic and enhance the bioactivity of other compounds.

Over the years, medicinal plants have been found useful in the treatment and management of various health problems. About 80% of the world population relies on the use of traditional medicine, which is predominantly based on plant material. Scientific studies available on a good number of medicinal plants indicate that promising phytochemicals can be developed for many health problems.[1] Plants produce diverse range of bioactive molecules, making them a rich source of different types of medinines. A rich heritage knowledge to preventive and curative medicines was available in ancient scholastic works included in the Atharvaveda, Charaka, Sushruta etc. over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry.[2]

Inflammation is typically viewed as a localized protective response to tissue damage and/or microbial invasion, which serves to isolate and destroy the injurious agent and the injured tissue and to prepare the tissue for eventual repair and healing. The survival value of the inflammatory response for both the injured tissue and the animal as a whole is evidenced by the fact that deficiencies of inflammation compromise the host and suggests that inflammation is an important physiological process. In most instances, an inflammatory reaction is short-lived and results in the desired protective response. However, in some cases, excessive and/or prolonged inflammation can lead to extensive tissue damage, organ dysfunction, and mortality.[3]

The critical role of inflammation in diseases as diverse as atherosclerosis, diabetes, cancer, reperfusion injury, and Alzheimer's disease accounts for the massive research effort that has been directed toward understanding the mechanisms that initiate and regulate the inflammatory response. While the molecular and cellular processes that contribute to inflammation remain poorly understood, advancements in this field of medical research have already facilitated the prevention, control, and cure of different human diseases associated with inflammation. Furthermore, the participation of a variety of signaling pathways, chemical mediators, and cell populations in the inflammatory response provides numerous potential targets for development of novel therapeutics for inflammatory diseases. Currently used synthetic anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary from medicinal plants origin. [4]

Tephrosia villosa belongs to family Fabaceae, commonly known as Nuguvemapalli in telugu and *Tephrosia villosa* in English. It is one of the most important plants used in the traditional system of medicine. The leaf juice is used to treat dropsy and diabetes. The roots and leaves contains rotenoids. *Tephrosia villosa* is being used extensively in preparation of many ayurvedic formulation for infection diseases, bacterial, microbial, diuretic etc. It consists of number of active chemical constituents. Our present study is to carry out the phytochemical screening of the leaf extract of *Tephrosia villosa* and also aimed to carry out the pharmacological evaluation of anti inflammatory activity of leaf extract of *Tephrosia villosa.*

Figure 1: *Tephrosia villosa*

2. Materials and Methods

Collection of plant material

Tephrosia Villosa plant leaves were collected from Tirumala hills, Chittor Dt., A.P. and authenticated by botanist Dr. K. Madhava Chetti, Asst. Professor, Dept. of Botany, S.V. University, Tirupathi and voucher specimen was deposited in S.V. University Botany Dept., Tirupathi.

Extract Preparation

The whole plant was allowed to dry under shade. The dried plant was powdered in a wiley mill. 100gm of dry powder was macerated with petroleum Ether (60-80^{0}c) for 12 hrs and refluxed for 3 hrs and then filtered and subjected to distillation under reduced pressure. The procedure was repeated for three times. Then the powder was air dried and again macerated with Ethanol for 12 hrs and it was refluxed for 3 hrs and then filtered and followed by distilled under reduced pressure to get semi solid mass and it is stored in desiccator. The procedure was repeated for three times to obtain residue which will be used for phytochemical studies and pharmacological studies.[5]

Preliminary Phytochemical analysis

The ethanol extract was subjected to preliminary phytochemical screening for the presence of phytoconstituents.

Qualitative method of phytochemical screening

The *Tephrosia villosa* leaf extracts were analyzed for alkaloids, flavanoids, glycosides, phenols, saponins, lipids and fat, tannins, anthraquinones, quinines, cardiac glycosides, coumarines, acids, steroids, phytosterols, proteins, carbohydrates etc.[6]

Detection of Alkaloids: About 50 mg of Solvent free extract was stirred with 3 ml of dilute hydrochloric acid and then filtered thoroughly. The filtrate was tested carefully with various alkaloid reagents as follows:

Mayer's test:
To a 1 ml of filtrate, few drops of Mayer's reagent are added by the side of the test tube. The white or creamy precipitate indicated test as positive.

Wagner's test: To a 1 ml of filtrate, few drops of Wagner's reagent are added by the side of the test tube. The color change was observed. A reddish-brown precipitates confirms the test as positive.

Dragendorff's test: To a 1 ml of filtrate, 2 ml of Dragendorff's reagent are added and the result was observed carefully. A prominent yellow precipitate confirms the test as positive

Detection of Carbohydrates

Fehling's test: One ml of extract was boiled on water bath with 1 ml each of Fehling solutions A and B. The color change was observed. A red precipitates indicated presence of sugar.

Barfoed's test: To 1 ml of extract, 1 ml of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. The color change was noted and recorded. A red precipitates indicated presence of sugar.

Benedict's test: To 0.5 ml of extract, 0.5 ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2minutes and the result was observed. A red precipitates indicated presence of sugar.

Detection of Glycosides

Legal's test: Chloroform (3ml) and ammonia solution (10%) was added to 2ml plant extract. Formation of pink color indicated the presence of glycosides.

Detection of Proteins:
The extract was dissolved in 10 ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate is subjected to tests for proteins and amino acids.

Million's test:
To 2 ml of filtrate, few drops of Millon's reagent are added. The result was observed. A white precipitates indicated presences of proteins.

Biuret test: An aliquot of 2 ml of filtrate was treated with drop of 2% copper sulphate solution. To this, 1 ml of ethanol (95%) was added, followed by excess of potassium hydroxide pellets. The pink color in ethanol layer indicated presences of proteins.

Detection of amino acid

Ninhydrin test: Two drops of ninhydrin solution (5 mg of ninhydrin in 200 ml of acetone) are added to two ml of aqueous filtrate. The color change was observed. A characteristic purple color indicated the presence of amino acids.

Detection of Phytosterols

Liberdmann-Burchard's test: The extract (5 mg) was dissolved in 2 ml acetic anhydride and one or two drops of concentrated sulphuric acid was added slowly along the sides of the test tube. The formation of blue green color indicated the presence of triterpenoids and phytosteroids.

Detection of Tannins

Ferric chloride test:
The extract (5 mg) was dissolved in 5 ml of distilled water and few drops of neutral 5% ferric chloride solution were added. The formation of blue green color indicated the presence of tannins.

Detection of Phenols

Lead acetate test: The extract (5 mg) was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitates indicated the presence of phenols.

Detection of flavonoids
An aqueous solution of the extract was treated with ammonium hydroxide solution. The yellow fluorescence indicated the presence of flavonoids.

Detection of coumarines
10% NaOH (1ml) was added to 1 ml of the plant extracts formation of yellow color indicated presence of coumarines.

Detection of Saponins
Distilled water 2ml was added of each plant extracts and shaken in a graduated cylinder for 15 mins lengthwise. Formation of 1cm foam indicates the presence of saponins.

Detection of Quinones
Concentrated sulphuric acid (1ml) was added to 1ml of each of the plant extract. Formation of red color indicated the presence of Quinones.

Detection of Cardiac glycosides
Glacial acetic acid (2ml) and few drops of 5% ferric chloride were added to 0.5% of the extract. This was under layered with 1ml of concentrated sulphuric acid. Formation of brown ring at the interface indicated presence of cardiac glycosides.

Detection of Terpenoids
Chloroform (2ml) and concentrated sulphuric acid was added carefully to 0.5 ml of extract. Formation of red brown color at the interface indicated the presence of terpenoid.

Detection of Anthraquinones
Few drops of 2% HCL were added to 0.5 ml of seed extract. Appearance of red color precipitate indicated presence of anthraquinones.

Detection of steroids and Phytosteroids
To 0.5 ml of the plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicated the presence of phytosteroids.

Pharmacological studies

Experimental animals
Colony inbred strains of male wistar rats weighing 150-180g were used for the pharmacological studies. The animals were kept under standard conditions (day/night rhythm) 8.00 am to 8.00 pm, 22 ± 10C room temperature, in polypropylene cages. The animals were fed on standard pellet diet and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions. It was randomly distributed into four different groups with six animals in each group under identical conditions throughout the experiments. The animals were maintained in accordance with CPCSEA guidelines. All the procedures described were reviewed and approved by Institutional Animal Ethical Committee.

Acute toxicity studies: The acute oral toxicity studies were carried out following OECD 423 guidelines.[7]

Anti-inflammatory activity:

The anti-inflammatory effect was evaluated by different doses of ethanolic extract of leaf of *T.villosa* using Carrageenan induced paw edema method.[8]

Treatment Protocol

Group: I: The animals received normal saline and served as normal control.

Group: II The animals received carrageenan and Standard drug Diclofenac sodium (10 mg/kg (p.o)) suspended in saline

Group: III The animals received carrageenan and ethanolic extract of Tephrosia villosa (200 mg/kg (p.o)) suspended in saline

***Group:* IV** The animals received carrageenan and ethanolic extract of Tephrosiavillosa (400mg/kg (p.o)) suspended in saline. The percent inhibition of paw edema volume is calculated using the formula, Percent inhibition =1-Yt/Yc x100 Where, Yt= Average increase in paw volume in groups tested with test compounds.

Yc= Average increase in paw volume in control

Statistical Analysis:

The statistical data was presented as mean ± SEM. Parametric data which include all the biochemical parameters were analysed using a paired "t "test for the paired data followed by one way analysis of variance (ANOVA). A probability value of P<0.001 was considered as significant.

3. Results and Discussions

Preliminary Phytochemical Studies: Ethanol extract gave positive tests for the alkaloids, carbohydrates, flavonoids, tannins, phenols, saponins, glycosides, terpenoids and steroids (Table-1).

Pharmacological Studies

Acute toxicity studies: Ethanol extract of leaf of *Tephrosia Villosa* was found to be safe since no animal died even at the maximum dose of 2000 mg/kg body weight.

Anti-inflammatory activity by carrageenan induced paw edema in rats: Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. [9] Effects of ethanolic extract of *Tephrosia Villosa* and Diclofenac sodium on carrageenan induced paw edema in rats were shown in Table-2.

Oral administration of the ethanolic extract at doses 200 and 400 mg/kg significantly suppressed the paw edema at 3 and 4 h after carrageenan injection in rats. Diclofenac sodium at a dose of 25mg/kg, significantly suppressed paw edema at 3 and 4 h after carrageenan administration (Table 2). In the control group, paw edema volume was maximum at the fourth hour. The test drugs showed dose dependent activity. The dose of 400 mg/kg p.o. exerted significant percentage of inhibition in edema volume (Table-2).

Discussion

The present study was undertaken on leaf of *T.villosa*. Anti-inflammatory activity was examined by carrageenan model. The carrageenan paw inflammation has been accepted as a useful diagnostic tool for investigation of systemic anti-inflammatory activity for any drugs. Ethanolic leaf extract of *T.villosa* showed dose dependent and significant inhibitory activity in carrageenan induced paw inflammation at 4th hour. The mechanism of action of carrageenan induced paw edema is described as biphasic. The first phase is due to the release of histamine, 5–HT and kinins in the first hour injection of carrageenan, the second phase is related to the release of prostaglandins like substance in 4th hour. Since, leaf extract of *T.villosa* reduced inflammation at 4^{th} hour, the biological compounds present in the leaf acted against prostaglandins like substances.

Table 1: Chemical tests carried out for ethanol extract of leaf of ***TephrosiaVillosa***

Chemical constituent	Name of the test	Pet.ether Extract	Ethanolic Extract
Alkaloids	Dragendroffs test	-Ve	+Ve
Carbohydrates	Molishs Test	-Ve	+Ve
Amino acids	Ninhydrin test	-Ve	-Ve
Steroids	Libermann Burchardtest	+Ve	+Ve
Flavonoids	Shinoda test	-Ve	+Ve
Tannins	Lead Acetate test	-Ve	+Ve
Phenol	$Fecl_3$ Test	-Ve	+Ve
Saponins	Froath test	-Ve	+Ve
Glycosides	Borntraggers test	-Ve	+Ve
Terpenoids	Hirschosin reaction	+Ve	+Ve

Table 2: *In-vitro* anti-inflammatory activity of ethanolic leaf extract of *Tephrosia villosa*

Drugs	Dose (mg / kg. p.o)	Paw edema volume at different time interval (in ml)				
		1 h	2 h	3 h	4 h	% inhibition
Vehicle	-	0.416±0.055	0.624±0.024	0.628±0.034	1.492±0.048	0
Diclofenac sodium	25	1.204±0.69	0.814±0.040	0.685±0.026	0.618±0.02	69.3***
TVEE	200	1.228±0.048	1.038±0.051	0.931±0.014	0.843±0.134	53.9***
TVEE	400	1.284±0.014	0.832±0.034	0.690±0.012	0.669±0.024	62.4***

Each value represents the mean ± SEM of 6 animals in each group***;*** ***p<0.001 when compared to Group-I

4. Conclusion

From the result, 400 mg/kg p.o. showed maximum percentage of inhibition in edema volume in carrageenan induced model, which might due to the higher concentration of active compounds involved in inhibiting prostaglandin synthesis. Further works, are needed to confirm the maximum activity of leaf of *T.villosa.* Moreover, some of the active compounds such as flavonoids, steroid compounds and anti-oxidants like oleic acid and palmitic acid took responsible for this pharmacological action and also need to be identified.

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