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Evaluation of the Ethanolic Leaf Extract of Tephrosia Villosa as an Anti-inflammatory Agent

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ABSTRACT

Tephrosia villosa, a member of the Fabaceae family, is a prominent plant in traditional medicine systems. The anti-inflammatory effects of its ethanolic leaf extract were investigated using a Carrageenan-induced inflammation model. In this study, inflammation was induced by subcutaneously injecting 0.1 ml of a freshly prepared 1% carrageenan solution in 0.9% sodium chloride. The test drugs were administered orally at doses of 250 and 450 mg/kg one hour before the experiment began. The anti-inflammatory activity was measured by comparing the paw volume (in ml) of animals treated with the test drugs to those in the vehicle control group. Diclofenac sodium (25 mg/kg orally) served as a reference drug. Results showed that the ethanolic leaf extract of T. villosa significantly reduced inflammation compared to the vehicle control. Diclofenac sodium also significantly reduced inflammation when compared to the control group. Among the two doses of T. villosa, the 450 mg/kg dose exhibited the most pronounced anti-inflammatory effect. These findings suggest that T. villosa leaf extract has notable anti-inflammatory properties, with the higher dose proving to be more effective.

Keywords: Tephrosia Villosa, Anti-Inflammatory, Traditional Medicine

Introduction

Tephrosia villosa (L.) Pers., a member of the Fabaceae family, is a perennial herb known for its extensive use in traditional medicine systems across various cultures. This plant, also commonly referred to as "villous tephrosia," has been employed in indigenous practices for its purported therapeutic benefits, including its role in treating inflammatory conditions. The therapeutic potential of T. villosa has garnered attention due to its rich phytochemical profile, which is believed to contribute to its medicinal properties.[1]

The leaves of T. villosa are particularly noteworthy for their diverse array of phytochemicals, including flavonoids, tannins, alkaloids, and saponins. These compounds are known for their potential pharmacological activities, including anti-inflammatory, antioxidant, and antimicrobial effects. Flavonoids and tannins, in particular, are compounds often associated with anti-inflammatory activity due to their ability to inhibit pro-inflammatory mediators and oxidative stress.[2]

Throughout history, medicinal plants have proven valuable for treating and managing a variety of health conditions. Approximately 80% of the global population depends on traditional medicine, which is largely based on plant-derived substances. Research on numerous medicinal plants has shown that they harbor promising phytochemicals that could address a range of health issues. Plants produce a wide array of bioactive compounds, making them a rich source of diverse medicinal agents.[3] Ancient texts, such as

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the Atharvaveda, Charaka, and Sushruta, contain extensive knowledge on both preventive and curative treatments. Furthermore, over half of all modern clinical drugs originate from natural products, highlighting their crucial role in pharmaceutical drug development.

Inflammation is generally recognized as a localized protective mechanism in response to tissue injury or microbial invasion. It aims to isolate and eliminate the harmful agent and damaged tissue, and to prepare for healing and repair.[4] The evolutionary significance of inflammation is underscored by its essential role in survival; deficiencies in this process can compromise the host's defense. Typically, inflammation is a temporary response that provides the necessary protection. However, in some cases, excessive or prolonged inflammation can cause significant tissue damage, organ dysfunction, and even death.

The significant role of inflammation in a wide range of diseases, including atherosclerosis, diabetes, cancer, reperfusion injury, and Alzheimer's disease, has led to extensive research aimed at understanding the mechanisms that trigger and regulate the inflammatory response.[5] Despite ongoing studies, many of the molecular and cellular processes underlying inflammation remain inadequately understood. Nevertheless, advancements in this area of medical research have already contributed to the prevention, management, and treatment of various inflammation-related diseases. The involvement of numerous signaling pathways, chemical mediators, and cell types in the inflammatory process offers many potential targets for developing new therapeutic approaches for inflammatory conditions. Current synthetic anti-inflammatory drugs often come with significant side effects, highlighting the need for the development of effective anti-inflammatory drugs derived from medicinal plants that offer fewer adverse effects.[6,7]

Ethanolic extraction is a common method used to isolate bioactive compounds from plant materials. Ethanol is an effective solvent for extracting a broad spectrum of phytochemicals, including those that are crucial for anti-inflammatory activity. The ethanolic leaf extract of T. villosa is expected to concentrate these active compounds, providing a robust preparation for evaluating its pharmacological effects. By using ethanol as the extraction solvent, the resultant extract is anticipated to contain a diverse range of bioactive constituents that can be evaluated for their anti-inflammatory properties.[8]

Materials & Methods

Extract Preparation

The extraction process began by drying the whole plant under shade. Once dried, the plant material was ground into a fine powder using a Wiley mill. A 100-gram portion of this dry powder was then subjected to an extraction process using petroleum ether (60-80°C). The powder was macerated in the petroleum ether for 12 hours, followed by refluxing for an additional 3 hours. The mixture was then filtered, and the solvent was removed via distillation under reduced pressure. This extraction procedure was repeated three times. Following this, the remaining plant powder was air-dried and then macerated with ethanol for 12 hours. The ethanol extraction was followed by refluxing for 3 hours, after which the mixture was filtered and the ethanol was evaporated under reduced pressure to yield a semi-solid mass. This semi-solid extract was stored in a desiccator. The ethanol extraction was also repeated three times to ensure thorough extraction. The resulting residue was used for subsequent phytochemical and pharmacological studies.

Preliminary Phytochemical Analysis

The ethanol extract of Tephrosia villosa leaves was subjected to preliminary phytochemical screening to identify various phytoconstituents. This qualitative analysis aimed to detect the presence of a range of chemical compounds including alkaloids, flavonoids, glycosides, phenols, saponins, lipids, fats, tannins, anthraquinones, quinones, cardiac glycosides, coumarins, acids, steroids, phytosterols, proteins, and carbohydrates.

Detection of Alkaloids

For the detection of alkaloids, approximately 50 mg of the solvent-free extract was dissolved in 3 ml of dilute hydrochloric acid. This solution was then filtered. The resulting filtrate was tested with various alkaloid reagents to identify the presence of alkaloids. This step is crucial in determining the phytochemical profile of the extract, as alkaloids are known for their diverse pharmacological activities.

- **Mayer's Test:** Add a few drops of Mayer's reagent to 1 ml of filtrate along the side of the test tube. The formation of a white or creamy precipitate indicates a positive result.
- **Wagner's Test:** Introduce a few drops of Wagner's reagent to 1 ml of filtrate along the side of the test tube. Observe for a color change. The appearance of reddish-brown precipitates confirms a positive result.
- **Dragendorff's Test:** Add 2 ml of Dragendorff's reagent to 1 ml of filtrate and observe the result carefully. The formation of a prominent yellow precipitate indicates a positive test.

Detection of Carbohydrates

- **Fehling's Test:** Boil 1 ml of extract with 1 ml each of Fehling's solutions A and B on a water bath. Observe for a color change. The appearance of red precipitates indicates the presence of sugar.

- **Barfoed's Test:** Add 1 ml of Barfoed's reagent to 1 ml of extract and heat in a boiling water bath for 2 minutes. Record any color change. Red precipitates indicate the presence of sugar.
- **Benedict's Test:** Add 0.5 ml of Benedict's reagent to 0.5 ml of extract and heat in a boiling water bath for 2 minutes. Observe the result. The formation of red precipitates indicates the presence of sugar.

Detection of Glycosides

- **Legal's Test:** Add 3 ml of chloroform and 10% ammonia solution to 2 ml of plant extract. The formation of a pink color indicates the presence of glycosides.

Detection of Proteins

Dissolve the extract in 10 ml of distilled water and filter through Whatman No.1 filter paper. Subject the filtrate to tests for proteins and amino acids.

- **Millon's Test:** Add a few drops of Millon's reagent to 2 ml of filtrate and observe the result. The formation of white precipitates indicates the presence of proteins.
- **Biuret Test:** Treat 2 ml of filtrate with a drop of 2% copper sulfate solution. Add 1 ml of ethanol (95%) followed by an excess of potassium hydroxide pellets. The formation of a pink color in the ethanol layer indicates the presence of proteins.

Detection of Amino Acids

- **Ninhydrin Test:** Add two drops of ninhydrin solution (5 mg of ninhydrin in 200 ml of acetone) to 2 ml of aqueous filtrate. Observe for a color change. The appearance of a characteristic purple color indicates the presence of amino acids.

Detection of Phytosterols

- **Libermann-Burchard's Test:** Dissolve 5 mg of extract in 2 ml of acetic anhydride and slowly add one or two drops of concentrated sulfuric acid along the sides of the test tube. The formation of a blue-green color indicates the presence of triterpenoids and phytosterols.

Detection of Tannins

- **Ferric Chloride Test:** Dissolve 5 mg of extract in 5 ml of distilled water and add a few drops of neutral 5% ferric chloride solution. The formation of a blue-green color indicates the presence of tannins.

Detection of Phenols

- **Lead Acetate Test:** Dissolve 5 mg of extract in distilled water and add 3 ml of 10% lead acetate solution. The formation of a bulky white precipitate indicates the presence of phenols.

Detection of Flavonoids

Treat an aqueous solution of the extract with ammonium hydroxide solution. The appearance of yellow fluorescence indicates the presence of flavonoids.

Detection of Coumarins

Add 1 ml of 10% NaOH to 1 ml of plant extract. The formation of a yellow color indicates the presence of coumarins.

Detection of Saponins

Add 2 ml of distilled water to each plant extract and shake in a graduated cylinder for 15 minutes lengthwise. The formation of 1 cm of foam indicates the presence of saponins.

Detection of Quinones

Add 1 ml of concentrated sulfuric acid to 1 ml of plant extract. The formation of a red color indicates the presence of quinones.

Detection of Cardiac Glycosides

Add 2 ml of glacial acetic acid and a few drops of 5% ferric chloride to 0.5 ml of extract. Underlay this mixture with 1 ml of concentrated sulfuric acid. The formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Detection of Terpenoids

Add 2 ml of chloroform and concentrated sulfuric acid carefully to 0.5 ml of extract. The formation of a red-brown color at the interface indicates the presence of terpenoids.

Detection of Anthraquinones

Add a few drops of 2% HCl to 0.5 ml of seed extract. The appearance of a red color precipitate indicates the presence of anthraquinones.

Detection of Steroids and Phytosteroids

Add an equal volume of chloroform to 0.5 ml of plant extract and add a few drops of concentrated sulfuric acid. The appearance of a brown ring indicates the presence of steroids, while a bluish-brown ring indicates the presence of phytosteroids.

Pharmacological Studies

Anti-inflammatory Activity

The anti-inflammatory effect was evaluated by administering different doses of ethanolic extract of Tephrosia villosa leaves using the Carrageenan-induced paw edema method.

Treatment Protocol

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

- **Group II:** The animals received carrageenan and the 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 Tephrosia villosa (400 mg/kg p.o.) suspended in saline.

The percent inhibition of paw edema volume was calculated using the formula:

Percent inhibition=(1−YtYc)×100\text{Percent inhibition} = \left(1 - \frac{Y_t}{Y_c}\right) \times 100Percent inhibition=(1−YcYt)×100

Where:

- YtY_tYt = Average increase in paw volume in groups tested with test compounds.
- YcY_cYc = Average increase in paw volume in the control group.

Statistical Analysis

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

Preliminary Phytochemical Studies

The ethanol extract of Tephrosia villosa leaves tested positive for alkaloids, carbohydrates, flavonoids, tannins, phenols, saponins, glycosides, terpenoids, and steroids.

Pharmacological Studies

- **Acute Toxicity Studies:** The ethanol extract of Tephrosia villosa leaves was found to be safe, as no animals 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 response of living tissues to injury. Steroidal anti-inflammatory agents can lyse and induce the redistribution of lymphocytes, causing a rapid and transient decrease in peripheral blood lymphocyte counts and affecting long-term responses.

The effects of the ethanolic extract of Tephrosia villosa and Diclofenac sodium on carrageenan-induced paw edema in rats. Oral administration of the ethanolic extract at doses of 250 and 450 mg/kg significantly suppressed paw edema at 3 and 4 hours after carrageenan injection. Diclofenac sodium at a dose of 25 mg/kg also significantly suppressed paw edema at 3 and 4 hours after carrageenan administration.

In the control group, paw edema volume was maximal at the fourth hour. The test drugs exhibited dose-dependent activity, with the 450 mg/kg dose exerting a significant inhibition of edema volume.

Discussion

This study investigated the anti-inflammatory activity of Tephrosia villosa leaves using the carrageenan model, which is widely accepted for evaluating systemic anti-inflammatory activity. The ethanolic leaf extract of Tephrosia villosa demonstrated significant dose-dependent inhibitory activity in carrageenan-induced paw inflammation at the fourth hour.[9,10]

The mechanism of action of carrageenan-induced paw edema is described as biphasic. The first phase, occurring within the first hour of carrageenan injection, is due to the release of histamine, serotonin (5-HT), and kinins. The second phase, occurring around the fourth hour, is related to the release of prostaglandin-like substances. The reduction of inflammation by the leaf extract of Tephrosia villosa at the fourth hour suggests that the bioactive compounds in the leaves act against prostaglandin-like substances.[11,12,13]

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