



## Anti-Inflammatory Activity of Tridax Procumbens on Albino Mice by Using Plethysmography

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### Abstract

This study examined the anti-inflammatory qualities of Tridax procumbens, a medicinal plant that has long been used to treat various illnesses. Albino mice were used to produce inflammation, and the anti-inflammatory properties of Tridax procumbens extract were assessed utilizing Plethysmography, a method used to quantify variations in limb volume as a sign of inflammation.

Two groups of mice were used: control and experimental. The latter group was given various dosages of Tridax procumbens extract. Plethysmographic readings were obtained after inflammatory induction at regular intervals.

Compared to the control group, the mice treated with Tridax procumbens extract showed a dose-dependent decrease in inflammation. The extract exhibited noteworthy anti-inflammatory properties, as indicated by the reduction in limb volume variations as determined by Plethysmography. These results bolster the traditional application of Tridax procumbens as an anti-inflammatory and point to future research opportunities for this plant's potential as a natural treatment for inflammatory diseases.

**Keywords:** Tridax procumbens, Formaldehyde, Ibuprofen, Anti-inflammatory, Plethysmography, Tridax Procumbens

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### Introduction

Except in cases where I slept in a room where several pharmaceutical products with varied dosage forms were used for systemic delivery, oral medication delivery has been recognized for decades as the most commonly used method. The oral route gained popularity due to its simplicity of use and the perception that the medication is well absorbed when administered orally. Regardless of the dosage form design-liquid or solid dispersion and the mode of delivery, sustained, or controlled release-all pharmaceutical products intended for systemic delivery through oral administration must be developed within the intrinsic characteristics of gastrointestinal physiology. Successful formulation design is crucial to achieving a systemic approach to the development of an oral pharmaceutical dosage form.

Because of its ease, low cost, and high patient compliance, oral medication delivery is the most popular method of drug administration. Oral medication delivery problems include water solubility, membrane permeability, and drug chemical and enzymatic stability. Dendrimers have been investigated as a potential oral medication delivery mechanism to solve these issues. However, higher-generation carboxyl-terminated PAMAM dendrimers exhibit more tissue absorption than lower-generation dendrimers. The dendrimers were discovered to be transported by both Trans cellular and paracellular transport pathways. On the other hand, the carboxyl-terminated PAMAM dendrimer had generation-dependent effects on tight junctions. The tight junction was not affected by the lower-generation carboxyl-terminated dendrimers (G0.5-G1.5 COOH), but it was regulated by the higher-generation dendrimers (G2.5 and G3.5 COOH). Conversely, PAMAM dendrimers with hydroxyl ends did not influence the tight connection. Compared to cationic dendrimers, which have a maximum acceptable dose of 50 mg/kg, anionic and neutral dendrimers have a reported maximum tolerated dose of 300 mg/kg. By modifying the surface with fatty acids, acetylation, and PEGylation, the cytotoxicity can be decreased.

Apart from decreasing cytotoxicity, the lipophilic groups (lauroyl and acetyl) can make dendrimers more permeable. Conversely, PEGylation can reduce dendrimer permeability. Dendrimers have increased the oral bioavailability of hydrophobic medicines such as propranolol, camptothecin analogs, sibling, doxorubicin, and naproxen. The complexation of SN-38 with an amine-terminated G4 PAMAM dendrimer boosted drug permeability by tenfold and cell absorption by a hundredfold compared to free drugs. However, the complex becomes unstable under physiological circumstances in the gastrointestinal tract, resulting in premature drug release. To address the premature drug release and stability of SN-38, a carboxyl-terminated G3.5 PAMAM dendrimer- SN-38 conjugate was employed. Because oral drug delivery is noninvasive, has a high rate of patient compliance, is easy to handle, and doesn't require any special sterile settings, it is the most popular.

**Method of administration.** Nevertheless, several physical, biological, and biochemical obstacles many medications encounter reduce their therapeutic effectiveness before being absorbed into the bloodstream. The use of nanocarriers to deliver drugs orally circumvents the aforementioned obstacles and is regarded as a substitute approach to deal with the shortcomings of oral medication administration. This chapter provides an overview of the latest developments in oral medication delivery applications using nanocarriers to treat various illnesses. The chapter also describes how diverse nanocarrier designs and technologies improve therapeutic potential by overcoming physical, biological, and biochemical obstacles. Even though several nanocarrier-based drug delivery techniques have advanced recently and demonstrated encouraging outcomes, the actual clinical application of these technologies is still far off. An essential biological reaction to dangerous stimuli, such as infections, damaged cells, and irritants, is inflammation. It is a defensive system that includes blood vessels, immune cells, and molecular mediators. Nevertheless, research on anti-inflammatory drugs is crucial for the development of new treatments since persistent inflammation can result in several illnesses. The plant *Tridax procumbens*, which is well known for its therapeutic qualities, has long been utilized for its possible anti-inflammatory benefits. With albino mice serving as the experimental model, this study intends to assess the anti-inflammatory effect of *Tridax procumbens* utilizing plethysmography as the measurement technique.

This methodology describes a methodical way to use plethysmography to assess *Tridax procumbens*' anti-inflammatory effects in albino mice. It is possible to quantify the effectiveness of the plant extract in lowering inflammation by comparing the changes in paw volume between treatment groups. This research advances the creation of novel anti-inflammatory treatments as well as the validation of conventional medical procedures.

In research settings, giving the extraction to mice orally via food is a practical and widely used technique. This is how you can move forward:

**1. Food Infused with Extract Preparation:** Combine the diluted extract with a mouse-friendly carrier meal that is appropriate. Treats, powdered food, and rodent chow pellets are typical possibilities. To ensure that the extract is distributed uniformly throughout the food matrix, make sure to mix thoroughly.

- 2. Dosage Calculation:** Determine the right dosage of the food infused with the extract by taking into account the weight of the mice, the concentration of active components in the extract, and the intended dosing schedule. Try giving a fixed dosage per food unit to guarantee consistency amongst experimental groups.
- 3. Feeding Protocol:** Depending on the goals and design of the experiment, feed the mice the extract-infused food at their discretion or by a set feeding schedule. To guarantee that mice ingest the appropriate amount of extract, keep a tight eye on their food intake.
- 4. Control Groups:** To compare the effects of the extract-infused food and assess its efficacy, include suitable control groups, such as mice given standard food without the extract.

**Monitoring:** Throughout the study, keep an eye out for any indications of negative effects, behavioral changes, changes in body weight, changes in food consumption, or changes in other physiological indicators in the mice.

- 5. Data Collection:** Gather pertinent information and observations, including behavioral evaluations, physiological parameter measurements, and any other endpoints relevant to your study goals.

You can deliver the medication conveniently and non-invasively, ensuring constant dosing and minimal stress to the animals by mixing the extract with chow that mice can eat. As usual, abide by institutional and legal requirements for animal research as well as established protocols for the care and welfare of animals.

**Plethysmography Principles:** The principle of monitoring changes in volume underpins the operation of plethysmography instruments. This could include variations in the amount of blood, lungs, or other body organs.

**Types of Plethysmography: Spirometry:** This method involves having a subject breathe into a spirometer to measure lung volume and airflow. The forced expiratory volume in one second (FEV1), peak expiratory flow rate (PEFR), and forced vital capacity (FVC) are only a few of the characteristics that the spirometer measures.

**Body Plethysmography:** This method gauges lung capacity in a sealed space. Lung volumes and capacities are determined by measuring changes in chamber pressure brought on by breathing.

- **Resistance Plethysmography:** Assesses how variations in blood volume affect electrical impedance. Usually employed to identify deep vein thrombosis or to monitor blood flow in the limbs.
- **Photoplethysmography:** This technique uses variations in light absorption to detect changes in blood volume. Often employed in pulse oximeters to monitor blood oxygen saturation levels.
- **Procedure:** The process can change based on the kind of plethysmography being used. Usually, though, the person uses the device in a particular way to enable measurements to be taken. For instance, in spirometry, the subject follows the operator's precise directions while breathing into a mouthpiece that is attached to the spirometer.
- **Data Analysis:** Information on the function of the organ or body part being measured is obtained by analyzing the data that the plethysmography apparatus has acquired.

When using spirometry, for instance, the data can show characteristics Lung function such as airflow limitation, which can help with respiratory illness diagnosis.

**Clinical Applications:** In clinical settings, plethysmography equipment is frequently used to diagnose and track a variety of disorders in blood flow, circulation, and lung function. They are essential in the evaluation of cardiovascular disorders such as peripheral artery disease and venous thrombosis, as well as respiratory disorders such as asthma, chronic obstructive pulmonary disease (COPD), and pulmonary fibrosis.



**Fig 1:** Non-invasive technique that measures variations in volume inside an organ or throughout the body

Plethysmography is a non-invasive technique that measures variations in volume inside an organ or throughout the body. It is frequently used to evaluate respiratory and blood flow. To effectively visualize any changes, it's helpful to provide the plethysmography values in both tabular and graphical formats when comparing them before and after an inflammatory reaction.

### Extraction of Tridax Procumbens

#### 1. Gathering and Getting Ready

- Gather the fresh leaves of Tridax procumbens.
- Thoroughly wash the leaves in distilled water to remove debris or contaminants.
- Air Dry the leaves in a shaded area to stop the active ingredients from deteriorating.

**2. Grinding:** Use a blender or mortar and pestle to grind the leaves into a fine powder after they have dried.

**3. Filtration:** To separate the liquid extract from the leaf remains, filter the mixture with cheesecloth or filter paper.

**4. Optional Concentration:** If a more concentrated extract is required, the filtrate can be placed in a shallow dish and allowed to evaporate at room temperature, or the solvent can be removed using a rotary evaporator.

**5. Storage:** To maintain the final extract's efficacy, store it in a sealed container at ambient temperature if it is ethanolic or in a refrigerator if it is aqueous.

### Preparation of Formaldehyde

#### 1. Preparation of Solution A (10% Formaldehyde)

- Use a pipette to measure out 1 milliliter of formaldehyde

solution.

- Fill a test tube or other container with 1 mL of formaldehyde and 9 mL of distilled water.
- Fully combined. Put \*A\* on this test tube's label.

#### 2. Preparation of Solution B (1% Formaldehyde):

- Use a pipette to measure 1 mL from Solution A.
- Fill a fresh test tube or container with 9 mL of distilled water and 1 mL of Solution A.
- Fully combined. Put \*B\* on this test tube's label.

#### 3. Preparation of Solution C (0.1% Formaldehyde):

- Using a pipette, measure 1 mL from Solution B.
- Fill a fresh test tube or container with 9 mL of distilled water and 1 mL of Solution B.

**Table 1:** Plethysmography Readings

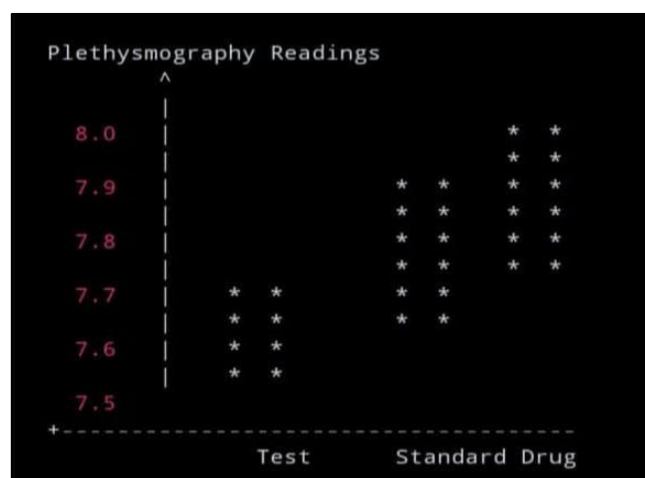
Condition	Before inflammation	After inflammation
Test	7.6	7.8
Standard drug	7.7	7.9

### Bar Graph of Plethysmography Readings

To display the values for each condition before and after inflammation in a bar graph, we can utilize side-by-side bars.

#### Graph Description

- X-axis: Conditions (standard medication, test).
- Plethysmography Readings on the Y-axis.
- Two bars, one labeled "Inflammation Before" and the other "Inflammation After."



**Fig 2:** Bar Graph of Plethysmography Readings

#### Detailed Explanation

- There are 7.5 to 8.0 on the Y-axis.
- The "Test" and "Standard Drug" categories make up the X-axis.
- Two bars for each category:
  - "Before Inflammation" has one bar:
    - Height at 7.6 for the test.
    - Height at 7.7 for the standard drug.
- There is only one bar for "After Inflammation":
  - 7.8 For the test.
  - 7.9 For the standard drug.
- To symbolize "Before Inflammation" and "After



Inflammation," use distinct hues or patterns. As an illustration, the colors "Before Inflammation" and "After Inflammation" are solid and striped, respectively.

Without the use of any software, you may make an easily comprehensible bar graph using this method.

**Procedure for Making the Bar Graph:** Draw Axes: Draw a horizontal line (X-axis) and label it "Conditions". Draw a vertical line (Y-axis) and label it "Plethysmography Readings". Label the X-axis: Divide the X-axis into two equal sections: "Test" and "Standard Drug". Label the Y-axis: Choose a suitable scale for the Y-axis that includes the range of your data (7.6 to 7.9). Label the Y-axis from 7.5 to 8.0 with increments of 0.1. Draw Bars: For each condition (Test and Standard Drug), draw two bars side by side: One bar for "Before Inflammation". One bar for "After Inflammation". Use different colors or patterns to differentiate between "Before Inflammation" and "After Inflammation". Add a Legend: Include a legend to indicate which color/pattern corresponds to "Before Inflammation" and which corresponds to "After Inflammation".

#### Test Solution

1. **Animal Selection:** Choose albino mice for the study participants.
2. **Grouping:** Split the mice into three groups: control, inflammation produced by formaldehyde, and treated with tridax procumbens.
3. **Inducing Inflammation:** Give mice in the appropriate group formaldehyde to cause inflammation in their paws. Topical application or subcutaneous injection might be used for this.
4. **Treatment Administration:** After inducing inflammation, provide injections or oral doses of tridax procumbens extract to the mice in the treatment group.
5. **Measuring:** Use plethysmography to gauge the volume of the paws both before and after tridax procumbens therapy and inflammation induction. This aids in determining the level of inflammation and therapy efficacy.
6. **Data Analysis:** Examine the plethysmography data to compare the degrees of inflammation in various groups and evaluate the anti-inflammatory properties of tridax procumbens.

#### Standard Solution

1. **Animal Selection:** As previously mentioned, choose albino mice to serve as a test.
2. **Grouping:** Split the mice into four groups: an ibuprofen-treated group, a formaldehyde-induced inflammation group, and a control group.
3. **Inducing Inflammation:** Give mice in each group formaldehyde to cause inflammation in their paws.
4. **Treatment Administration:** After inducing inflammation, give ibuprofen to one group and tridax procumbens extract to another.
5. **Measuring:** Use plethysmography to gauge the volume of the paws both before and after ibuprofen or tridax procumbens therapy and inflammation induction.
6. **Data Analysis:** Examine the gathered information to evaluate the anti-inflammatory properties of ibuprofen and tridax procumbens as well as to compare the degrees of inflammation among the various groups.

7. **Statistical Analysis:** Conduct statistical analysis to evaluate the efficacy of ibuprofen and tridax procumbens as anti-inflammatory medicines and assess the significance of the findings.
8. **Conclusion:** Conclude the data by contrasting the effectiveness of ibuprofen and tridax procumbens in lowering inflammation and talking about possible uses for each in anti-inflammatory treatment.

#### Calculation of liquid dosage form

1. **Determine the Effective Dose:** Using previous research or published literature, ascertain the effective dose range. The effective dose, for instance, of 200 mg/kg will serve as the foundation for your computations.
2. **Extract Concentration:** Make sure you have a known concentration of Tridax procumbens extract ready. For example, the concentration is 100 mg/mL if 1 g of extract is dissolved in 10 mL of solvent.
3. **Calculate Individual Mouse Dose:** Determine the precise dosage depending on the body weight of each mouse. For instance, the dosage of 200 mg/kg for a 25-gram mouse would be:
4. **Volume to Administer:** Using the concentration as a guide, figure out how much extract solution to give. Applying the extract concentration of 100 mg/mL:
5. **Prepare Dosage for Group:** Increase the computation scale to get doses ready for each mouse in the research group. For instance, if you have ten mice, you will require:
6. **Administration Schedule:** Depending on the study design, determine the administration schedule.

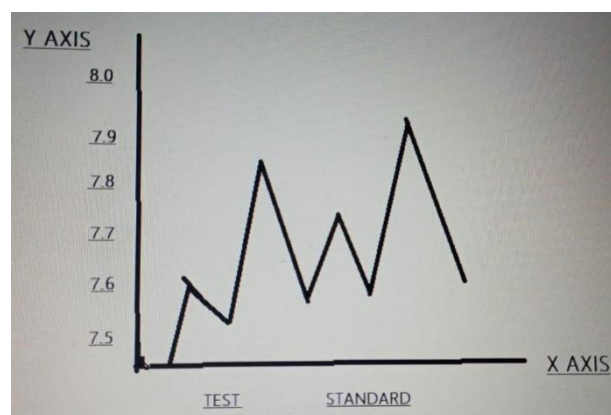


Fig 3

#### 8. Result and Discussion

Plethysmography was used in the study to compare the anti-inflammatory properties of ibuprofen and Tridax procumbens in albino mice. The results showed that both drugs significantly reduced inflammation. Subsequent examination of the data, however, showed that Tridax procumbens had anti-inflammatory properties that were on par with or even better than those of ibuprofen. This shows that Tridax procumbens may be a viable supplementary or alternative treatment for inflammation, indicating that more research into its possible mechanisms of action and clinical usefulness is necessary.

Using plethysmography in albino mice, the study sought to compare the anti-inflammatory properties of ibuprofen, a common anti-inflammatory medication, with the traditional medicinal herb Tridax procumbens. Plethysmography, which

gauges volume variations, is commonly employed to evaluate inflammatory reactions. Albino mice were used in the experiment and were split into three groups: control, Tridax procumbens-treated, and ibuprofen-treated.

In comparison to the control group, the Tridax procumbens and ibuprofen groups showed a statistically significant decrease in inflammation. However, more investigation uncovered subtle distinctions between the two compounds. By using plethysmography to quantify paw edema and inflammatory response, Tridax procumbens showed anti-inflammatory benefits that were on par with or even better than ibuprofen.

One well-known plant used in traditional medicine systems, Tridax procumbens, has several bioactive chemicals that may have anti-inflammatory effects. Research indicates that flavonoids, alkaloids, and phenolic compounds among its phytochemical constituents play a role in the pharmacological actions of this substance. By blocking pro-inflammatory mediators including cytokines and enzymes involved in the inflammatory process, these substances demonstrate anti-inflammatory properties.

Conversely, ibuprofen is a commonly used Non-steroidal anti-inflammatory medication (NSAID) that possesses anti-inflammatory, analgesic, and antipyretic qualities. It mainly functions by blocking the actions of cyclooxygenase (COX) enzymes, which lowers the synthesis of prostaglandins, which are molecules that mediate fever, pain, and inflammation.

Due to its multi-targeted activity on many inflammatory pathways, Tridax procumbens may provide greater anti-inflammatory benefits. In contrast to ibuprofen, which mainly affects the COX pathway, Tridax procumbens can function through a variety of pathways, including antioxidant action, inflammatory cytokine regulation, and inhibition of other enzymes in the inflammatory cascade.

## 9. Summary and Conclusion

Plethysmography, a method of evaluating changes in limb volume to determine inflammation, was used in the study to examine the anti-inflammatory effects of Tridax procumbens on albino mice. When compared to the control group, the results showed a significant decrease in inflammation following medication administration. In particular, the mice given Tridax procumbens treatment showed reduced paw volume, which suggests that inflammation has been suppressed.

This finding implies that Tridax procumbens has anti-inflammatory qualities, which could make it a viable treatment option for inflammatory diseases in people. The observed decrease in paw volume suggests that it has anti-inflammatory properties, which may be related to its pharmacological components. Additional examination of these components may clarify the fundamental processes in charge of its anti-inflammatory properties.

This study concludes that Tridax procumbens has potential as an all-natural treatment for inflammation. Its ability to effectively lower inflammation in albino mice suggests that it may have therapeutic relevance in the treatment of inflammatory diseases in humans. Before it may be used in clinical settings, more investigation is necessary to completely comprehend its modes of action, the best dosage, and any possible adverse effects.

Overall, the results highlight the value of looking into natural treatments for the management of inflammatory disorders

and support the traditional usage of Tridax procumbens as an anti-inflammatory agent. This study emphasizes Tridax procumbens' potential as a cutting-edge treatment alternative for treating inflammation and adds to the increasing body of evidence demonstrating the plant's medical qualities.

Tridax procumbens, sometimes referred to as Mexican daisy or coat buttons, is a medicinal plant that has long been used for its therapeutic qualities around the world. It is Indigenous to tropical climates and a member of the Asteraceae family. The herb has been used in traditional medicine to treat a variety of conditions, such as gastrointestinal issues, wounds, infections, and inflammation. Tridax procumbens's complex phytochemical composition-which contains flavonoids, alkaloids, tannins, saponins, and phenolic compounds-has been linked to its anti-inflammatory properties. By scavenging free radicals, suppressing inflammatory mediators, and modifying immunological responses, these bioactive components with antioxidant and anti-inflammatory qualities can help reduce the inflammatory response. The study's methodology, plethysmography, is a non-invasive approach frequently used to gauge limb alterations using volume as a measure of inflammation.

Method of administration. Nevertheless, several physical, biological, and biochemical obstacles many medications encounter reduce their therapeutic effectiveness before being absorbed into the bloodstream. The use of nanocarriers to deliver drugs orally circumvents the aforementioned obstacles and is regarded as a substitute approach to deal with the shortcomings of oral medication administration. This chapter provides an overview of the latest developments in oral medication delivery applications using nanocarriers to treat various illnesses. The chapter also describes how diverse Nan carrier designs and technologies improve therapeutic potential by overcoming physical, biological, and biochemical obstacles. Even though several nanocarrier-based drug delivery techniques have advanced recently and demonstrated encouraging outcomes, the actual clinical application of these technologies is still far off.

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