FORMULATION AND IN- VIVO EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF TRIDAX PROCUMBENS L. AND AZADIRACHTA INDICA EXTRACT IN CARRAGEENAN-INDUCED ALBINO WISTAR RATS

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Abstract

Traditional medicine systems like Ayurveda, Traditional Chinese Medicine, and Native American medicine have used natural resources for healing purposes for thousands of years. These systems have provided a foundation for modern pharmacology. Many modern pharmaceutical drugs have been developed from compounds initially isolated from plants. The Earth's biodiversity offers a vast array of potential medicinal compounds. However, a significant portion of these natural resources remains unexplored. This highlights the need for continued research into the pharmacognostic and pharmacological properties of various plants and organisms. The study aimed to evaluate anti-inflammatory activity and phytochemical screening of novel polyherbal formulations *Tridax procumbens* and *Azadirachta indica*. It was observed that Test group Seven had an ethanolic extract of *Tridax procumbens* and *Azadirachta indica.* (75:25) shows the best result like the standard drug Diclofenac Sodium. Test groups show dose-dependent therapeutic efficacy. Efficacy increases with increasing the dose of test formulations.

Keywords Albino wistar rats, anti-inflammatory, *Azadirachta indica*, carrageenan, treatment, *Tridax procumbens* L.

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Introduction

Inflammation is a natural response of the body to harmful stimuli, such as pathogens, injuries, or allergens. It serves as a defense mechanism to protect the body and initiate the healing process. It is essential for the body's defense, chronic or uncontrolled inflammation can contribute to a wide range of health problems. These include allergies, cardiovascular diseases, metabolic syndrome, cancer, and autoimmune diseases. Chronic inflammation can impose a significant economic burden on individuals and society due to healthcare costs and decreased productivity. Traditional medications like steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and immunosuppressants are commonly used to control and suppress inflammation. However, they may be associated with adverse effects, especially when used at high doses or for prolonged periods. Natural anti-inflammatory factors, including herbal medicines, are of interest because they may provide therapeutic benefits with fewer side effects. However, it's crucial to conduct scientific research to establish their safety and efficacy. Herbal medicines are often perceived as having a milder or gentler effect on the body. They work to support and uplift the body's natural processes that may have become deficient or imbalanced. They are known for helping the body eliminate excesses, such as toxins or imbalances, and have a holistic approach to healing, focusing on overall well-being and restoring equilibrium. Herbal medicines have been used for centuries in various cultures for their potential therapeutic properties. They can be a valuable source of natural anti-inflammatory agents. Scientific studies and clinical trials are necessary to validate their effectiveness and safety in specific medical contexts. Complementary, alternative, and traditional medicine systems often incorporate herbal remedies. These systems can provide valuable guidance, but rigorous scientific validation is essential before adopting herbal treatments into mainstream medical practice. Modern medicine relies on scientific methods and evidence-based practices. Before herbal medicines are widely recommended or prescribed, they should undergo rigorous scientific evaluation to determine their safety, efficacy, and appropriate dosage regimens [1-6].

Tridax procumbens (L.), belongs to the Asteraceae, commonly known as coat buttons or tridax daisy, which is a widely distributed weed. It is found in India, America, Tropical Africa, Asia, and Australia. It has become naturalized in various regions around the world. In traditional Indian medicine (Ayurveda), it has been used for liver disorders, gastritis, and heartburn. Additionally, it has been employed as an anticoagulant, and antifungal agent, and for treating infectious skin diseases. The plant contains various bioactive compounds, including alkaloids, steroids, carotenoids, flavonoids (such as catechins and flavones), saponins, and tannins. Organic solvent extraction with ethyl acetate has been found to yield flavonoids like centaureidin and centaurein, as well as bergenin. Secondary metabolites present in the plant include fatty acid derivatives, sterols, lipid constituents, luteolin, glucoluteolin, quercetin, isoquercetin, and fumaric acid. Pharmacologically it is reported as having hepatoprotective, immunomodulating Properties, wound healing activity, hypotensive effect, antimicrobial, anti-inflammatory, antioxidant, and insect repellent. It is also used as a bio-adsorbent to remove harmful Cr (VI) from industrial wastewater [7-15].

Azadirachta indica (Meliaceae), commonly known as neem, is indeed a versatile plant with a long history of traditional medicinal use. It is native to tropical and semi-tropical regions and is known for its various medicinal properties. Neem trees are abundant in tropical and semi-tropical regions. They are fast-growing and can reach substantial heights. The leaves of the neem tree are commonly used for medicinal purposes. Neem has a rich history of use in traditional medicine, particularly in the Indian subcontinent. It has been employed to treat a wide range of ailments, including skin conditions, digestive issues, and more. Different parts of the neem tree, including seeds, leaves, flowers, and bark, are utilized for various purposes in traditional medicine. It contains a wide range of phytochemicals, including quercetin, azadirachtin, limonoids (nimbin, nimbinin, nimbidin), nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, ascorbic acid, n-hexacosanol, nimbiol, amino acids, and other bioactive compounds. These compounds contribute to its medicinal properties. Its health-promoting properties, including antioxidant, anti-inflammatory, antimicrobial, and potential anticancer effects, have made it a valuable resource. Various products viz. neem oil and neem extracts, are widely used in modern healthcare and personal care products, including skin creams, shampoos, and toothpaste are already available in the market [16-.25]

Methodology

Materials and Method

The yield of powdered whole-plant of *Tridax procumbens* and *Azadirachta indica*, extracted using the Soxhlet technique by using ethanol as the solvent and the dark greyish brown ethanolic extract yielded 6.23% and 6.47%. From this yeild further study was done.

***Tridax procumbens and Azadirachta indica*: A Toxicity Analysis**

I. Acute oral toxicity study: Acute oral toxicity study were conducted according to OECD guideline 423 ANNEX 2c.

Study design: Three animals were selected for each group. Three polyherbal formulations is prepared by the ethanolic extract of *Tridax procumbens* and *Azadirachta indica.*

Test sample A: (50:50) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Test sample B: (25:75) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Test sample C: (75:25) ethanolic extract of *Tridax procumbens* and *Azadirachta*

II. Experimental Design for screening model of *Carrageenan induced Paw Oedema in rats*:

In this study, 48 mature albino Wistar rats were split into 8 groups of 6. The following distinctions were made in how these two groups were cared for:

Group 1: Inflammation induced vehicle group (0.1 ml carrageenan)

Group 2: Rats treated with 0.1 ml Carrageenan + Rats treated with Diclofenac sodium 10 mg/kg b.wt. and were kept as standard group

Group 3: Rats treated with 0.1 ml Carrageenan + 200 mg/kg (50:50) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 4: Rats treated with 0.1 ml Carrageenan + 2000 mg/kg (50:50) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 5: Rats treated with 0.1 ml Carrageenan + 200 mg/kg (25:75) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 6: Rats treated with 0.1 ml Carrageenan + 2000 mg/kg (25:75) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 7: Rats treated with 0.1 ml Carrageenan + 200 mg/kg (75:25) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 8: Rats treated with 0.1 ml Carrageenan + 2000 mg/kg (75:25) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

The dose level was used as follows:

Group 1: 200 mg/kg (50:50) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 2: 2000 mg/kg (50:50) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 3: 200 mg/kg (25:75) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 4: 2000 mg/kg (25:75) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 5: 200 mg/kg (75:25) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

Group 6: 2000 mg/kg (75:25) ethanolic extract of *Tridax procumbens* and *Azadirachta indica*

III. Experimental Procedure

An experimental setup involving 48 adult albino wistar rats to induce an inflammatory reaction. The rats fasted for 12 hours before the experiment. To induce an inflammatory reaction, the rats' paws were swollen with Carrageenan. After swelling the paws, the rats received a single intraperitoneal injection of Carrageenan at a dose of 1 ml per kilogram of the rat's body weight. Intraperitoneal injection involves injecting the substance into the peritoneal cavity, which is the space in the abdominal area. After sterilizing the injection site, the designated amount of Carrageenan was injected into the peritoneal cavity.

Carrageenan Induced Inflammation

Acute paw oedema was induced by injecting 1% w/v carrageenan (0.1 ml) into the sub plantar region of the left hind paw in all animal groups. A digital Plethysmometer was used to measure the volume of each paw at the beginning, middle, and end of the first, second, third, and fourth hours. At 4 hours, we assumed a level of inflammation in Group-1 (the vehicle group) of 100% and estimated the percentage of oedema inhibition."

IV. Experimental detail:

Method : Carrageenan induced paw oedema in rats(1 ml/kg, i.p.)

Animal used : Albino wistar rats Weight : 150-200 gms

No. of group : Eight

Route of administration : P.O.

Standard drug used : Diclofenac Sodium (10 mg/kg body weight, orally)

Drug Profile

Sr. No.	Drugs	Dose
1	Carrageenan	1%/ kg b.w (0.1 ml)
2	Diclofenac Sodium	10 mg/kg

Working Procedure of Plethysmometer

- A worksheet that details the experiment day, the study's title, a list of the animals involved, and the conditions under which inflammation is to be induced has been produced in advance.
- Make sure that the environmental temperature is kept constant.
- Adjust the mercury level if required.
- Mark the paw with the ink at the level of lateral malleolus
- Immerse the paw in the mercury up to this mar k.
- Note the increase in mercury level at the graduated side of the plethysmograph.
- Always dip the paw up to the ink mark for accurate results.
- Remove the paw and take extra precautions for cleaning the paw because if the traces of mercury are licked by the animal, death may occur.
- One of the paws is regarded as a control paw.
- Repeat the experiment at different time intervals.
- Clean the instrument and adjust the mercury level after completion of experiment.

Result & Discussion

The evaluation of the acute oral toxicity and anti-inflammatory effects of aqueous and ethanolic leaf extracts of *Tridax procumbens* (L.) and *Azadirachta indica* on albino Wistar rats, conducted in accordance with OECD 423 criteria. The study found that even at a high dosage of 2000 mg/kg/p.o. (oral administration), there were no fatalities or serious adverse responses observed in the rats. Therefore, the LD_{50} (lethal dose for 50% of the test subjects) for both aqueous extracts of *Tridax procumbens* and *Azadirachta indica* was determined to be 2000 mg/kg/p.o., indicating that these extracts are safe at this dosage. It also involve in the induction of inflammation using carrageenan, a commonly used inflammatory agent in various groups. For which, Group I, which received carrageenan induction, showed significant increases in paw volume, indicating inflammation. Groups 2 and 7, which received carrageenan induction and either a standard drug (10 mg/kg) or an ethanolic extract blend of Tridax procumbens and Azadirachta indica (75:25 ratio) (200 mg/kg/p.o.), exhibited the greatest reduction in inflammation after a 4-hour period. Paw volume decreased in these groups, indicating anti-inflammatory effects. Groups 3, 4, 5, 6, and 8, which received carrageenan induction followed by oral dosing of ethanolic extracts of Tridax procumbens and Azadirachta indica at different doses (200 and 400 mg/kg/p.o.), also showed significant reductions in paw edema. The second and seventh groups had the highest percentages of paw edema inhibition, with rates of 98.14% and 96.47%, respectively, indicating strong anti-inflammatory effects whereas the fifth group had the lowest inhibition rate at 92.69%. Overall, the study suggests that both *Tridax procumbens* and *Azadirachta indica*, especially in ethanolic extract form, possess significant anti-inflammatory effects, as demonstrated by their ability to reduce paw edema in carrageenan-induced inflammation models. Additionally, the extracts were found to be safe when administered orally at a dosage of up to 2000 mg/kg/p.o., as indicated by the absence of fatalities or serious adverse responses in the rats.

Conclusion

The purpose of this research was to analyze the new polyherbal formulation of *Tridax procumbens* and *Azadirachta indica* (Neem) for its anti-inflammatory efficacy. Both the plants were used to create polyherbal preparations, which were then tested for their anti-inflammatory efficacy. It was shown that the ethanolic extract of *Tridax procumbens* and *Azadirachta indica* (75:25). in Test Group Seventh exhibited the best anti-inflammatory results. These results were comparable to those of the standard drug Diclofenac Sodium. This indicates that the polyherbal formulation has significant anti-inflammatory potential. As the dose of the test formulation increased, so did its efficacy in reducing inflammation. This suggests that higher doses may yield stronger anti-inflammatory effects. The study identified specific chemical constituents, namely terpenoids (such as nimbin and sodium nimbidate) and flavonoids (like ferulic acid and caffeic acid), as responsible for the anti-inflammatory activity observed in the polyherbal formulation. This formulation could offer an accessible and cost-effective option for managing inflammation, particularly in regions where these plants are abundant. However, further research and clinical trials may be necessary to validate

these findings and establish the safety and efficacy of the polyherbal formulation for broader use.

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