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RESEARCH ARTICLE
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EVALUATION OF *AZADIRACHTA INDICA* LEAVES EXTRACT FOR ANTI-ALLERGIC POTENTIAL

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Abstract
This study investigates the antiallergic potential of *Azadirachta indica* leaves extract as a natural remedy for allergic conditions. *Azadirachta indica*, commonly known as Neem, has a history of medicinal use in traditional medicine systems. The ethanolic extract was prepared from the leaves and evaluated in allergen-induced allergic reactions using animal models and *in-vitro* process. The extract demonstrated significant antiallergic effects, reducing histamine release, suppressing pro-inflammatory cytokines, and enhancing immunomodulatory activity. These findings support the traditional use of *Azadirachta indica* and its potential as an antiallergic agent. The presence of bioactive compounds like nimbin, nimbidin, and azadirachtin may contribute to its antiallergic properties. Further research is needed to elucidate the underlying mechanisms and evaluate the extract's safety and efficacy in human subjects. If proven effective, *Azadirachta indica* leaves extract could offer an affordable and accessible alternative for managing allergic conditions.

Keywords: Azadirachta indica, allergy, ethanolic, nimbin, histamine

Introduction

Allergic disorders are a significant global health concern, affecting a substantial portion of the population worldwide. These conditions encompass a wide spectrum of immune-mediated hypersensitivity reactions, ranging from mild irritations to severe, life-threatening anaphylaxis. The pathophysiology of allergies involves an exaggerated immune response to typically harmless

substances, known as allergens, leading to the release of inflammatory mediators such as histamine, leukotrienes, and cytokines. This cascade of immune reactions results in the manifestation of various allergic disorders, including asthma, allergic rhinitis, atopic dermatitis, and food allergies. (1, 2, 3)

Conventional treatments for allergies often involve the use of antihistamines, corticosteroids, and immunomodulatory drugs. While effective in managing symptoms, these medications may carry adverse effects and are not always well-tolerated by all individuals. Therefore, there is a growing interest in exploring alternative, natural remedies for allergic conditions to complement existing therapies and improve overall patient outcomes. (4, 5, 6)

Azadirachta indica, commonly known as neem, is a traditional medicinal plant with a long history of therapeutic use in various cultures. Its leaves are particularly rich in bioactive compounds, which have been studied extensively for their diverse pharmacological properties. Among these compounds, nimbin, nimbidin, quercetin, and azadirachtin have shown potent anti-inflammatory and antioxidant activities, making *Azadirachta indica* a promising candidate for its potential antiallergic effects.(7,8) The present study aims to evaluate the antiallergic potential of *Azadirachta indica* leaf extract using in vivo experimental models. By investigating the extract's ability to modulate key inflammatory pathways and immune responses associated with allergies, we seek to provide scientific evidence for its therapeutic efficacy in alleviating allergic reactions. (9)

The need for this study arises from the increasing prevalence of allergic disorders worldwide and the limited availability of safe and effective natural antiallergic treatments. The potential of *Azadirachta indica* as a plant-based remedy for allergic conditions presents an exciting opportunity to expand the therapeutic options available to allergy sufferers. (10)

Materials and Methods

2.1 Collection of Plant Material

The *Azadirachta indica* (AI) leaves were carefully collected from the local area and subjected to authentication by a renowned botanist. A voucher specimen was prepared and preserved for future reference, ensuring the accuracy and reliability of the plant material used in the study.

2.2 Preparation of ethanolic Extract:

The leaves were cleaned by washing with running water and shade dried and the milled to pass through 100-mesh sieve. The leaf powder was extracted by maceration for three days with 80% ethanol at room temperature. The extracts were concentrated at 45^0 C using Rotary vacuum evaporator to yield 80% fraction. The concentrated extracts were keep in refrigerator at 4°C until further use.

2.3 Phytochemical Screening:

The ethanolic leaves extract of *AI* underwent phytochemical analysis to determine the presence of various compounds. The analysis aimed to identify the existence of volatile oils, carbohydrates, alkaloids, glycosides, polyphenols, flavonoids, tannins, propanoids, sterols, terpenoids, ketones, and alcohols in the extract.

2.4 Experimental Animals

Albino Wistar mice weighing between 25-30g were chosen as subjects for the experimental study. The animals were housed in an animal facility approved by the Institutional Animal Ethics Committee (IAEC-CPCSEA) and maintained under standard laboratory conditions. The laboratory environment was set at a temperature of 22 ± 2℃, relative humidity of 50 ± 15%, and a 12-hour light/dark cycle. Throughout the study, the rats had unrestricted access to standard pellets as their food and water was provided *ad libitum.*

2.5 Methodology

2.5.1 Assessment of Antiallergic Activity using isolated goat tracheal chain preparation (11,12)

This method was used to assess direct antihistaminic activity of plant extract. The mechanism by which the epithelium affects the reactivity of tracheal musculature can be studied using the isolated perfused trachea preparation. The dose relative contractile responses are observed for different agonists like Ach, histamine, 5-HT, bradykinin and their antagonists on goat trachea. Goat trachea brought from slaughter house was cut into individual rings and tied together in a series to form a chain. It was suspended in organ bath containing Kreb's solution, maintained at 37 ± 1 ^{0}C, a stream of 5% CO_2 in oxygen was bubbled through organ tube. One end tied to aerator tube and other attached to isotonic frontal writing lever to smoked drum. Tissue was allowed to equilibrate for 45 min under a load of 200-400 mg. A dose response curve (DRC) for histamine (100 µg/ ml) was taken in variant molar concentrations. A time cycle of 5 min was followed. DRC of histamine in absence and presence of Diphenhydramine (1 µg/ml, 0.1 ml) and extracts (25 mg/ml, 0.5 ml) were recorded (n = 5). Graph of maximum percentage of contractile response on ordinate and log molar concentration of histamine on abscissa was plotted to record DRC of histamine.

2.5.2 Clonidine-induced catalepsy (13, 14)

Clonidine causes mast cell degranulation in brain, causing histamine release in higher amount. This will causes imbalance between dopaminergic systems. This will ultimately leads to cataleptic condition. Drugs having antihistaminic property will reduce the cataleptic duration. Mice were divided in six groups. Clonidine (1 mg/kg, s.c.) was injected to mice pretreated with Group 1 served as control (10 ml/kg, p.o.), Group 2 as Diphenhydramine (1mg/kg., i.p.), and Group 3 to Group 5 treated with extracts (200 and 400 mg/kg). The forepaws of mice were placed on a horizontal bar (1 cm in diameter, 3 cm above the table). The time required to remove the paws from bar was noted for each animal. Duration of catalepsy was measured at 0, 30, 60, 90, 120, 150 and 180 min interval.

2.5.3 Statistical Analysis

All observations were presented as mean ± SEM. The data was analyzed by student's t-test (unpaired) and one-way ANOVA followed by Dunnett's test. $p < 0.05$ was considered as significant. Prism graph Pad 5 was used for statistical analysis.

3.0 Results

3.1 Acute toxicity study

The oral administration of ethanolic extracts of *A. indica* leaves up to 2000 mg/kg did not produce any toxic effects and no mortality was observed in mice. Results indicated that doses upto 2000 (mg/kg; p.o.) were non lethal. All animals were found to be alive but slightly sluggish during the observation period of 24 hours post administration of the highest dose.

3.2 Phytochemical screening

Phytochemical screening revealed the presence of flavonoids, tannins, saponins and terpenoids.

3.3 In-vitro activity:

3.3.1 Effect of Diphenhydramine on histamine-induced contraction of isolated goat tracheal chain preparation

A dose response curve (DRC) for histamine (100 mcg/ ml) was taken in variant molar concentrations. DRC of histamine in presence of aqueous solutions of Diphenhydramine (1 µg/ ml) were recorded.
Histamine produced a dose dependent contraction of isolated goat tracheal chain preparation. Diphenhydramine significantly ($P < 0.01$) inhibited histamine-induced contraction of goat tracheal chain preparation. The result is shown in fig 1

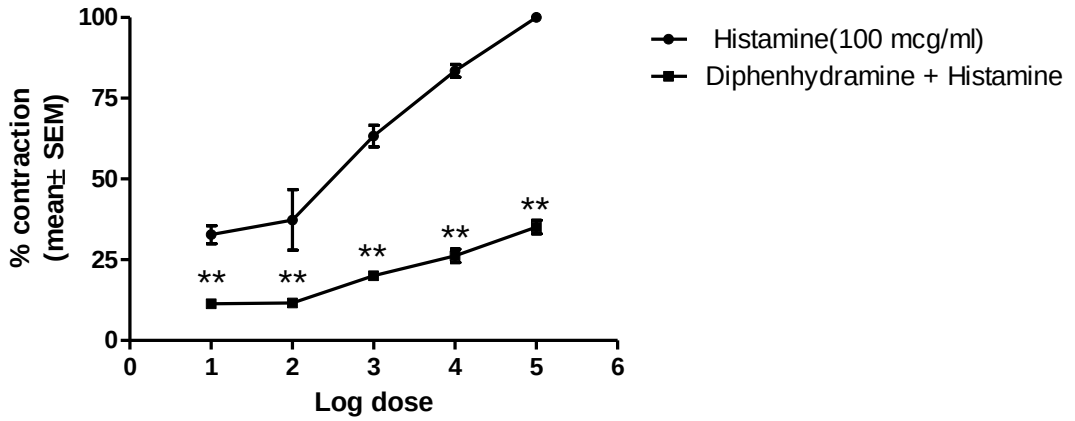


Figure 1: Effect of Diphenhydramine on histamine-induced contraction of isolated goat tracheal chain preparation

3.3.2 Effect of Ethanolic extract of *AI* on histamine-induced contraction of isolated goat tracheal chain preparation

A dose response curve (DRC) for histamine (100 mcg/ ml) was taken in variant molar concentrations. DRC of histamine in presence of ethanolic extract solutions were recorded.

Histamine produced a dose dependent contraction of isolated goat tracheal chain preparation. *AI* at different doses significantly ($P < 0.05$) inhibited histamine-induced contraction of goat tracheal chain preparation. In presence of the extract the agonist could not produce maximal effect. This indicates that extracts may have antihistaminic activity. The result is shown in fig 2.

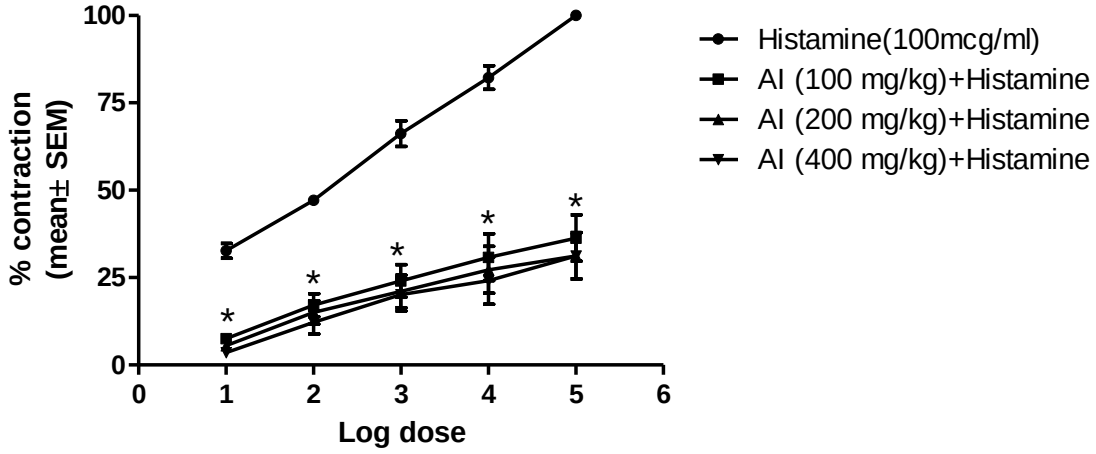
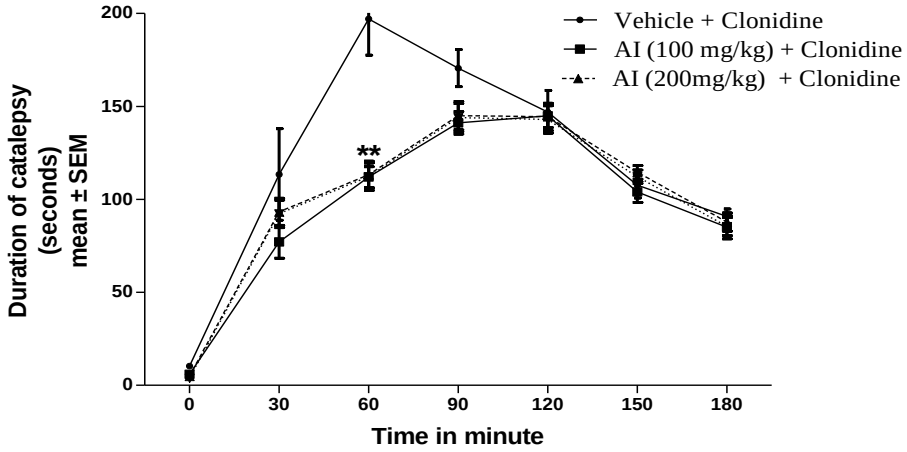


Figure 2 Effect of ethanolic extract of *AI* on histamine-induced contraction of isolated goat tracheal chain preparation

3.3.3 Effect of various doses of ethanolic extract of *AI* on Clonidine – induced catalepsy in experimental animals

The duration of catalepsy was increased in group 1 (control) after administration of Clonidine (1 mg/kg) subcutaneously in experimental animals. The duration of Clonidine induced catalepsy was decreased significantly ($p < 0.01$) in group 200 and 400 mg/kg at time 60 min as compared to group 1 (control). The results are depicted in fig 3



4.0 Discussion:

The findings from this study provide evidence supporting the antiallergic potential of ethanolic extract of *Azadirachta indica* leaves extract. The observed decrease in histamine release and modulation of pro-inflammatory cytokines indicates that the extract may suppress allergic reactions and alleviate symptoms associated with allergies. These results align with previous research on the anti-inflammatory and immunomodulatory properties of *Azadirachta indica*. (15, 16)

The presence of bioactive compounds in the *Azadirachta indica* leaves extract may be responsible for its antiallergic effects. Some of the compounds found in *Azadirachta indica*, such as nimbin, nimbidin, and azadirachtin, have been reported for their anti-inflammatory and immune-regulatory activities. These compounds may act synergistically to suppress immune hypersensitivity, thereby reducing allergic responses. (17)

The use of natural plant extracts like *Azadirachta indica* can be advantageous in antiallergic therapy due to their potential effectiveness and relatively lower risk of adverse effects compared to synthetic medications. Furthermore, plant-derived remedies may provide a more affordable and accessible option for individuals with allergic conditions. (18)

However, further research is warranted to fully understand the underlying mechanisms of action of *Azadirachta indica* leaves extract in antiallergic responses. Additional studies should explore the extract's safety profile, potential drug interactions, and long-term effects to establish its viability as a clinical antiallergic treatment. (19-57)

5.0 Conclusion

This study contributes to the growing body of evidence supporting the use of *Azadirachta indica* leaves extract as a potential antiallergic agent. The observed antiallergic effects and the presence of bioactive compounds in the extract warrant further investigation and clinical trials to determine its efficacy and safety in treating allergic conditions in humans. If proven effective and safe, *Azadirachta indica* leaves extract could be a valuable addition to the arsenal of natural remedies for allergic disorders.

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