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Antihistaminic effects of *Azadirachta indica* leaves in laboratory animals

Research Article

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Abstract

Azadirachta indica (Meliaceae) leaves have been traditionally used in the management of asthma and the current study was undertaken to scientifically validate the benefits of plant as an antihistaminic agent using the suitable animal model. The agents with antihistaminic properties are known to be good antiasthmatic agents; hence, in the current research work, the antihistaminic activity of an ethanolic extract of *Azadirachta indica* leaves (at a dose of 250 mg/kg, i.p.) was evaluated using haloperidol-induced catalepsy and clonidine-induced catalepsy in laboratory rats. The results showed that the ethanolic extract inhibits the catalepsy induced by the clonidine but no remarkable effect was observed on the catalepsy induced by haloperidol. This strongly suggests that, the inhibition is mediated through an antihistaminic action and there is no role of dopamine. Hence, in the present study, it is concluded that, the ethanolic extract has significant antihistaminic activity. The polar constituents in the ethanolic extract of leaves of *Azadirachta indica* may be responsible for the antihistaminic effects and therefore, the ethanolic leaves extract can be a better remedy as an antihistaminic agent.

Key Words: Asthma, Antihistaminic, *Azadirachta indica*, catalepsy bar, Haloperidol, Clonidine.

Introduction

Neem is a huge evergreen tree that can reach a height of 20 metres. The leaves are alternating, with 8-19 leaves per leaflet, and they appear in March-April. The leaves have a bitter taste (1).

Through the augmentation of antioxidant activity, suppression of bacterial growth, and manipulation of genetic pathways, plant products or natural products play an essential role in disease prevention and therapy. Because of their low side effects and low cost, the medicinal usefulness of a variety of plants in disease management is still being vigorously explored. It is widely acknowledged that allopathic medications are pricey and have a harmful effect on normal tissues and biological activity. The notion that many pharmacologically active medications are sourced from natural resources, including medicinal plants, is widely recognized (2, 3).

The Bible and the Quran, for example, both promote the use of herbs in health care and prevention. The function of herbs in disease management is also confirmed from an Islamic perspective, with Prophet Mohammed (PBUH) recommending several plants/ fruits for disease treatment (4). Many infectious, metabolic, and malignant illnesses are treated with neem components in Ayurveda, Unani, Homeopathy, and modern medicine (5, 6).

In many countries, several types of preparations based on plants or their elements are quite popular in illness management. Based on the fact that neem (Azadirachta indica), a member of the Meliaceae family typically found in India, Pakistan, Bangladesh, and Nepal, has therapeutic implications in disease cure and formulation. Azadirachta indica has a complex of compounds including as nimbin, nimbidin, nimbolide, and limonoids, which play a role in illness management by modulating multiple genetic pathways and other activities. Quercetin and ß-sitosterol were the first polyphenolic flavonoids isolated from fresh neem leaves, and they had antifungal and antibacterial properties (7).

Plant is also described to have antibacterial (8), antifungal (9) and anti-inflammatory (10) properties. Antiarthritic, antipyretic, hypoglycemic, antigastric ulcer, antifungal, antibacterial and anticancer properties have been established by previous researchers (10, 11, 12, 13) and a review described the many therapeutic roles of neem (14). The role of neem and its active components in disease prevention and therapy via modification of numerous biological pathways is summarized in this article.

Catalepsy is a condition in which an animal retains an imposed posture for an extended period of time before returning to its natural position. Catalepsy is

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a symptom of medications that block dopaminergic transmission or enhance histamine release in the brain having an extrapyramidal effect. Clonidine, a 2-adrenoceptor agonist, causes catalepsy in rat in a dose-dependent manner that is blocked by histamine H1-receptor antagonists but not by H2-receptor antagonists (15). In this study, we aimed to analyze the plant's antihistaminic activity in order to see if there is any evidence for its traditional use in asthma.

Materials and Methods

Plant material

The plant *Azadirachta Indica* was collected from Pune district (Maharashtra) in December, 2021, and authenticated by the Botanical Survey of India, Pune (Voucher specimen No. ANAI1), date of authentication was 17/01/2022.

Animals

Male Wistar albino rats weighing 180-200 g were randomized in to three groups and housed under standard laboratory conditions in groups of six each. The animals were provided ad. Libitum access to food and water. The protocol was approved by IAEC (Institutional animal ethical committee of Progressive Education Society's Modern College of Pharmacy, Yamunanagar, Nigdi, Pune-44).

Drugs and chemicals

The drugs used were: clonidine (Unimedicolab, Utttarakhand, India), haloperidol (RPG Life sciences Ltd., Gujrat, India.), and pheniramine maleate (Sanofi India Ltd., Ankleshwar, Gujrat, India); all the chemicals were purchased from a commercial source. Chemicals used were: ethanol AR (New Neeta Chemicals, India) and Tween 80 AR (New Neeta Chemicals, India).

Preparation of extract

In this process, 1000 g of the coarsely powdered leaves and seed was kept in a stoppered container with 1000 ml of ethanol and the solvent was kept in contact with plant material for the period of 3-days at room temperature, provided with intermittent agitation until the soluble matter dissolved. The resulting mixture was then filtered and the marc was pressed and the extract was clarified with filtration after standing. The yield of the extract was 12.63%

Phytochemical Analysis (16)

Phytochemical analysis was done as per the reported procedure.

Quantitative estimation of total phenolic and flavonoid content (16)

The extracts were quantified as per the reported procedure

Anticataleptic activity

Effect on clonidine-induced catalepsy

To study the effects of extract on clonidine induced catalepsy, the catalepsy bar test was used. Clonidine (1 mg/kg, s.c.) was injected into the rats (n=6) 30-min before the treatment with vehicle (Tween 80 in distilled water; max. upto 10 mL/kg, p.o.) and ethanol extract (250 mg/kg, i.p.) and standard drug pheniramine maleate (10 mg/kg, i.p.). The dose selection was made based on an acute toxicity study reported earlier (17). The forepaws of rat were placed on a horizontal bar (1-cm in diameter, 12 cm above the table) and the time consumed by the rat to withdraw its paws from the bar was recorded for each rat; the catalepsy duration was measured at 0, 15, 30, 60, 90, 120, 150, and 180 min.

Effect on haloperidol-induced catalepsy

The same protocol was followed with administration of haloperidol as an inducing agent. Haloperidol (1 mg/kg, i.p.) was injected to rats (*n*=6) before 30-minutes of administration with vehicle (Tween 80 in distilled water; Max. 10 mL/kg, p.o.) or ethanol extract (250 mg/kg, i.p.). The catalepsy duration was measured at 0, 15, 30, 60, 90, 120, 150, and 180 min.

Statistical Analysis

The data is presented as a mean + standard error of the mean (SEM). Two-way ANOVA was used to evaluate the data. For statistical analysis, Prism Graph Pad 8.4.3 was utilized. The significance level was set at ***P<0.001.

Results

A) Phytochemical analysis

Using conventional procedures, qualitative phytochemical testing of extracts was carried out to investigate the presence or absence of various phytochemical elements. Phytochemical analysis of several extracts revealed the presence of carbohydrates, flavonoids, phenolics, lipids and oils, saponins, and other compounds.

Phytochemical Constituents	Yes (+) / No (-)
Carbohydrates	+
Proteins and Amino acids	+
Flavonoids	+
Alkaloids	+
Triterpenoids	+
Tannins	+
Fat and oil	+
Steroids	+
Saponins	+
Glycosides	+

B) Quantitative estimation of total phenolic and flavonoid content

Total phenolic content of extracts of Azadirachta Indica Leaves	
Extract	Total phenolic content (mg GAE/g extract)
Ethanolic	13.68 ± 0.53

x represents quantity/concentration of phenols which is obtained from the equation: y=0.014×+0.103, where y is absorbance of samples and R^2=0.999.

Results are expressed in mean ± SD (n=3), SD: Standard deviation, A Indica: *Azadirachta Indica*, GAE: Gallic acid equivalents

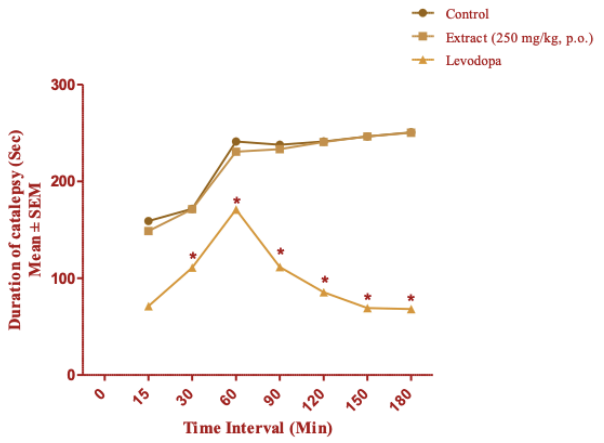
Total flavonoid content of extracts of Azadirachta Indica	
Extract	Total Flavonoid content (mg RE/g extract)
Ethanolic	524.08 ± 0.92

x represents quantity/concentration of flavonoids which is obtained from the equation: y=0.008×+0.180, where y=absorbance of samples and R^2=0.996. Results are expressed in mean ± SD (n=3), SD: Standard deviation, *A. Indica:* Azadirachta Indica

C) Anticataleptic activity

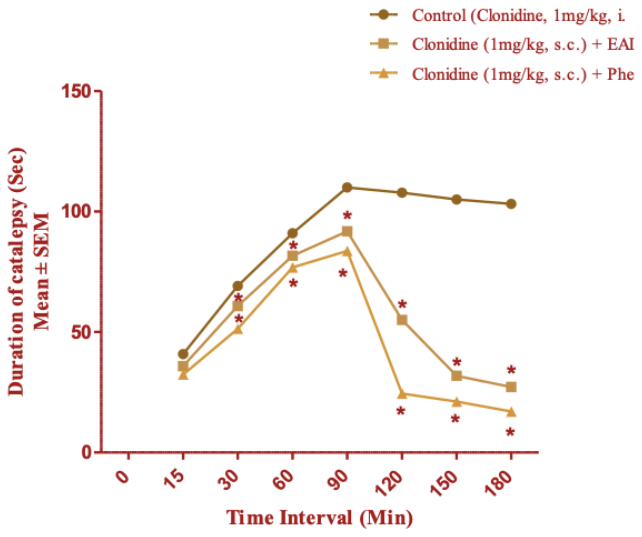
1) Effect on clonidine induced catalepsy

The data was analyzed by Two-way ANOVA followed by Bonferroni's posttest. * (P<0.001) as compared to control group.



The results show that the duration of catalepsy induced by clonidine was significantly (p<0.001) reduced by the treatment with extract at 250 mg/kg, i.p. The effects offered by the extract were found parallel to the standard drug Pheniramine (10 mg/kg, i.p.).

2) Effect on Haloperidol induced catalepsy



The data was analyzed by Two-way ANOVA followed by Bonferroni's posttest. * (P<0.001) as compared to control group.

There was no significant effect on duration catalepsy induced by haloperidol by treatment with extract (250 mg/kg, i.p.). In opposite to this, catalepsy duration was significantly reduced by the standard drug Levodopa

Discussion

It had been observed that, animals develop catalepsy as a result of side effect of a variety of medicines. Some researchers investigated the relative roles of acetylcholine and histamine in perphenazine-induced catalepsy and proposed that antidepressant's anticholinergic activity could be attributable to an increase in dopamine levels in the brain or their capacity to suppress acetylcholine release (18). Some investigators also discovered that the amount of histamine in the brain appears to be directly related to the stage of catalepsy. Uvnas investigated mast cell degranulation and its relationship to histamine release after a mast cell degranulating substance (Compound 48/80) was administered (19). Clonidine releases histamine from mast cells in a similar way to a selective liberator like compound 48/80, according to Lakdawala et al (20).

Pretreatment with L-histidine, a precursor of histamine, potentiated clonidine-induced catalepsy in a dose-dependent way, the researchers discovered. In conscious rat, intracerebroventricular injection of histamine caused catalepsy, which was prevented by H1-receptor antagonist but not by H2-receptor antagonist, according to Muley et al (21). Clonidine is known to release histamine from mast cells (20). In the brain, Schwatz discovered histamine-producing mast cells (22). Prazosin, a 2-adrenoceptor blocker, inhibits clonidine-induced histamine release from mast cells (23).

Neuroleptics cause catalepsy as well, but by a different mechanism: they block dopamine D2-receptors in the substantia nigra (24, 25). This study found that an ethanol extract of *A. Indica* leaves can prevent clonidine-induced catalepsy but not haloperidol-induced catalepsy. The cataleptic action of clonidine in rat is mediated by histamine release from mast cells, according to this study. This extract's ability to prevent clonidine-induced catalepsy is most likely owing to its mast cell-stabilizing properties. Dopaminergic transmission is not affected by the plant. As a result, the polar components could be employed as an antihistaminic and in asthma treatment.

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Conflict of Interest

Authors declare that, there is no conflict of interest.

References

1. Puri H. S, Neem: the divine tree Azadirachta indica. Harwood Academic Publishers. 1999; 9-21.
2. Zong A., Cao H, Wang F. Anticancer polysaccharides from natural resources: a review of recent research. Carbohydr. Polym. 2012; 90(4); 1395–1410.
3. Efferth T., Koch E. Complex interactions between Phytochemicals. The Multi-Target Therapeutic concept of Phytotherapy. Curr. Drug Targets. 2011; 12(1); 122–132.
4. Al-Bukhari M. I, Al-Bukhari S. The Collection of Authentic Sayings of Prophet Mohammad (Peace Be upon Him), Division 71 on Medicine. 2nd. Ankara, Turkey: Hilal Yayinlari; 1976.
5. Brahmachari G. Neem—an omnipotent plant: a retrospection. ChemBioChem. 2004; 5(4); 408–421.
6. Ketkar A.Y, Ketkar C.M, Jacobson M, Ketkar M. S. and Schmutterer, H. Various uses of neem products. The Neem Tree: Azadirachta indica A. Juss. and Other Meliaceous *Plants*. 1995; 518-558.
7. Govindachari T. R, Suresh G, Gopalakrishnan G, Banumathy B, Masilamani S. Identification of antifungal compounds from the seed oil of Azadirachta indica. Phytoparasitica. 1998; 26(2); 109–116.
8. Singh N, Sastry M. S. Antimicrobial activity of Neem oil. Indian Journal of Pharmacology. 1997; 13; 102–106.
9. Kher A, Chaurasia S. C. Antifungal activity of essential oils of three medical plants. Indian Drugs. 1997; 15; 41–42.
10. Bandyopadhyay U, Biswas K, Sengupta A. Clinical studies on the effect of Neem (Azadirachta indica) bark extract on gastric secretion and gastroduodenal ulcer.
    Life Sci. 2004; 75(24); 2867–2878.
11. Sultana B, Anwar F, Przybylski R. Antioxidant activity of phenolic components present in barks of Azadirachta Indica, Terminalia Arjuna, Acacia Nilotica, and Eugenia Jambolana Lam. trees. Food Chem. 2007; 104(3); 1106–1114.
12. Ebong P. E, Atangwho I. J, Eyong E. U, Egbung G. E. The antidiabetic efficacy of combined extracts from two continental plants: *Azadirachta indica* (A. Juss) (Neem) and *Vernonia amygdalina* (Del.) (African Bitter Leaf). Am J Biochem Biotechnol. 2008; 4(3); 239–244.
13. Paul R, Prasad M, Sah N. K. Anticancer biology of *Azadirachta indica* L (neem): a mini review. Cancer Biol. Ther. 2011; 12 (6); 467–476.
14. Biswas K, Chattopadhyay I, Banerjee R. K, Bandyopadhyay U. Biological activities and medicinal properties of neem (Azadirachta indica). Curr. Sci. 2002; 82(11); 1336–1345.
15. Jadhav J. H, Balsara J. J, Chandorkar A. G. Involvement of histaminergic mechanisms in the cataleptogenic effect of clonidine in mice. J Pharm Pharmacol. 1983; 35; 671-3.
16. Siddhu N, and J. Saxena. Quantification of total phenolic and total flavonoid content of extracts of tagetes erecta flowers. Asian J. Pharm. Clin. Res. 2017; 10(6); 328.
17. Braga, Teresa M., et al. Azadirachta indica A. Juss. In Vivo Toxicity—An Updated Review. Molecules. 2021; 26(2); 252.
18. Chopra Y. M, Dandiya P. C. The relative role of brain acetylcholine and histamine in perphenazine catatonia and influence of antidepressants and diphenhydramine alone and in combination. Neuropharmacol. 1975; 14; 555-60.
19. Uvans B. Mast cells and histamine release. Indian J Pharmacol. 1969; 1; 23-32.
20. Lakadwala A. D, Dadkar N. K, Dohadwala A. N. Action of clonidine on mast cells of rats. J Pharm Pharmacol. 1980; 32; 790-791.
21. Mulay M. P, Balsara J. J, Chandorkar A. G. Intarcerebroventricular administration of histamine in conscious mice during catalepsy. Indian J Pharmacol. 1979; 11; 277-81.
22. Schwartz J. C, H. W. Elliot, R. George, and R. Okun. Annu. Rev. Pharmacol. Toxicol. 1977; 325-339.
23. Weiner N. Norepinephrine, epinephrine and the sympathomimetic amines. The pharmacological basis of therapeutics. 1985:145-60.
24. Sanberg P. R. Haloperidol induced catalepsy is mediated by postsynaptic dopamine receptor. Nature. 1980; 284; 472-3.
25. Ossowska K, Karcz M, Wardas J. Straiatal and nucleus accumbens D1/D2 dopamine receptors in neuroleptic catalepsy. Eur J Pharmacol. 1990; 182; 327-34.

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