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Preventive Effect of an Extract of *Terminalia avicennioides* on Acetylcholine-Induced Tracheoconstriction in Wistar Strain Rats

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Abstract

Asthma is one of the most common chronic respiratory pathologies worldwide. The cost of its treatment, particularly for populations in developing countries like Senegal, requires an alternative which consists of developing research into new therapeutic approaches with the use of plants from traditional pharmacopoeia. The objective of this study was to determine whether the hydroalcoholic extract of *Terminalia avicennioides* leaves has a preventive effect on the constriction of tracheal smooth muscle induced by Acetylcholine in Wistar strain rats. Methods: The species used in our experiments were male Wistar rats aged between 12 and 14 weeks. Rings of rat trachea were suspended in isolated organ chambers connected to tension sensors. Two pharmacological agents were used: acetylcholine, for its constrictor effect and adrenaline, for its dilating effect on the pulmonary trachea. Our plant material consisted of a hydroalcoholic extract of *Terminalia avicennioides* leaves. The experimental protocol was that of the preventive model which consisted of impregnating these rings with the extract at a defined concentration then testing its contractility in the presence of Acetylcholine which was the contractile agonist used in this study. The preventive effect of the extract was studied with an Acetylcholine contraction range on rings pre-incubated with *Terminalia avicennioides* extract. Results: The results showed that Acetylcholine contracts the trachea and the increase in its contractility was dependent on the dose of Ach added cumulatively (10^{-5} to 4×10^{-5} mol/l). The administration of the hydroalcoholic extract of *Terminalia avicennioides* leaves led to

a modification of the contractile response to Acetylcholine. Indeed, this extract induced a significant attenuation of the contractile responses of the treated tracheal rings compared to those untreated. Conclusion: *Terminalia avicennioides* had a relaxing effect on tracheal smooth muscle and this effect could play an important role in the prevention of bronchial hyperreactivity in certain respiratory diseases, particularly asthmatic disease.

Keywords

Bronchial Hyperreactivity, Extract Hydroalcoholic of Terminalia Avicennioides, Asthma

1. Introduction

Respiratory diseases are a public health priority according to the World Health Organization (WHO). Asthma is one of the most common chronic respiratory diseases worldwide [1] [2]. It represents a significant cause of morbidity in developed countries due to its high prevalence, which has tended to increase in recent decades, and a major concern in developing countries for economic and humanitarian reasons [3] [4] [5]. Asthma affects about 350 million people in the world, its frequency has been steadily rising for 40 years and its mortality remains worrying, with more than 250,000 deaths per year worldwide [6]. According to WHO estimates, the burden of the disease accounts for 1% of the total burden induced by all diseases globally [7].

According to a group of international experts, asthma is "a chronic inflammatory disorder of the airways in which several types of cells and their mediators are involved. Chronic inflammation is associated with airway hyperresponsiveness, which leads to recurrent episodes of wheezing, dyspnea, chest tightness, and/or cough, particularly at night or early in the morning. These episodes are often associated with extensive obstruction of variable degree, which is often spontaneously reversible or responsive to treatment" [7]. In fact, bronchial hyperresponsiveness (BHR), which is one of the main components of asthma, is characterized by an abnormal susceptibility of the bronchi to contract excessively in response to stimuli that have no effect on a normal subject [8]. The prevention and treatment of BHR is an endlessly pursued goal. Alongside modern medicine treatments, the use of medicinal plants is a common practice in the management of BHR in our traditional African societies [9] [10] [11].

Traditional Senegalese pharmacopoeia is very rich, and many plants are used by traditional practitioners to treat respiratory diseases. However, in Senegal, the efficacy of these plants has not been scientifically proven, and information on effective doses and potential toxicity is not available.

In our study, we aim to verify the pharmacological efficacy of *Terminalia avicennioides* leaves by testing the degree of attenuation of contractile responses

induced by the studied extract at the level of the isolated rat trachea *in vitro*.

2. Methodology

2.1. Plants Material

Our plant material consisted of *Terminalia avicennioides* leaves, which had been dried for a week to facilitate their conservation and use during grinding, which allows the plant to be reduced to very fine particles but also allows obtaining a powder of low tenacity, facilitating the extraction process. From the obtained powder, we prepared the crude hydroalcoholic extract.

One hundred (100) grams of powder were macerated in an Erlenmeyer flask with 300 ml of 60% MeOH and 200 ml of distilled water for 2 hours, taking care to stir the flask every 15 minutes. Subsequently, the macerate was subjected to vacuum filtration using a Kitassato flask on a funnel and then on hydrophilic cotton to obtain the hydroalcoholic extract. The evaporation of the hydroalcoholic extract was carried out using a rotary evaporator until a dry residue was obtained under the following evaporation conditions: bath temperature at 50˚C, cooling at 21˚C, rotation at 4000 rpm.

After evaporation, the obtained dry residues had to be protected from light and humidity (T < 25˚C) at a temperature equal to 4˚C. Our extract was the one prepared from *Terminalia avicennioides* leaves. We took 10 mg of dried sample in a dry tube to homogenize in 10 ml of distilled water or Krebs saline solution which resulted in a dilution 10 mg/10 ml.

2.2. Solvents and Pharmacological Agents

Solvents: It is essential to preserve the muscle reactivity of the trachea during its removal to optimize its motor function. Indeed, biological phenomena were highly influenced by external conditions such as temperature, pH, ionic concentrations, etc.

To study the different physiological phenomena *in vitro*, it was important to maintain these different factors so that the biological functions studied were preserved. This is why we used Krebs solution, which was a physiological liquid providing the essential nutrients for maintaining these different physiological functions, as well as the different ions involved in its metabolism. Krebs solution was also used to fill the cuvettes during the dilution of different preparations of pharmacological substances, but also during the removal of the trachea. The extraction solvents used were methanol, distilled water, and anhydrous trifluoroacetic acid (TFA).

Pharmacological agents: We mainly used two pharmacological agents, acetylcholine for its constrictor effect on the pulmonary trachea and adrenaline for its dilating effect on the pulmonary trachea.

2.3. Experimental Animals

The species used in our experiments were the male Wistar rat, aged between 12

and 14 weeks with a weight of 300 to 350 grams. The animals were kept in plastic cages with iron mesh and measuring 42 cm long by 27 cm wide and 15 cm high. The cages had a litter that was frequently renewed. The rats were kept in a well-ventilated environment close to thermal neutrality, between 25˚C and 30˚C. The rats had access to water through a drinking bottle, and their diet consisted mainly of maize grains and fish flour.

- **Withdraw of cervical trachea**

Tracheal rings were obtained after anesthetizing the rat with an anesthetic (3% chloral) intraperitoneally. Then, the cervical trachea was dissected, first the animal is exsanguinated by cutting with curved scissors at the level of the abdominal aorta, then the cervical trachea was removed. The collected fragment was placed in a petri dish and kept constantly immersed in Krebs solution to prevent drying. The fatty adhesions were removed under a binocular loupe, by tearing the fatty tissue with fine forceps and scissors. Finally, the trachea was then cut into rings of 3 to 4 mm in length using scissors.

- **The experimental protocol**

The experimental protocol was that of the preventive model, which consisted of impregnating a fragment of the trachea with the extract at a defined concentration and then testing its contractility in the presence of acetylcholine, which was the contractile agonist used in this study. Thus, we proceeded as follows: An equilibration step consisted of mounting the tracheal ring on the metal rod that was immersed in the cuvette containing Krebs solution, stabilizing at a base tension of 1.5 g for 45 minutes by renewing the Krebs every 10 minutes; performing the electrical zero before proceeding to tracheal reactivity tests. For the functional test, we sensitized the trachea with 10µ of acetylcholine concentrated at 10^{-1} g/l, after rinsing we performed a contraction with acetylcholine at the same previous concentration, then a relaxation of the trachea with adrenaline at 10^{-1} mol/l. This last test proved the presence of the receptors involved. Our work consisted of creating a contraction curve of the trachea using increasing doses of acetylcholine ranging from 10^{-5} to 4×10^{-5} mol/l.

Indeed, in each cuvette containing 10 ml of Krebs solution, we successively injected the following volumes of acetylcholine to obtain the respective final concentrations in the cuvette:

- 10 µ of 10^{-1} mol/l acetylcholine corresponds to 10^{-5} mol/l in the cuvette.
- 10 µ of 10^{-1} mol/l acetylcholine corresponds to 2×10^{-5} mol/l in the cuvette.
- 10 µ of 10^{-1} mol/l acetylcholine corresponds to 3×10^{-5} mol/l in the cuvette.
- 10 µ of 10^{-1} mol/l acetylcholine corresponds to 4×10^{-5} mol/l in the cuvette.

After administering each dose of Ach, we waited for the curve to reach the plateau before administering the next dose. Then, we washed the tracheal rings by renewing the Krebs solution in the cuvette several times. After returning to the base tension, the rings were incubated with the extract at a concentration of 1 g/l for 30 minutes before proceeding to another contraction with increasing doses of acetylcholine. The obtained contractions were amplified and recorded

using a data acquisition system.

2.4. Study Framework

All manipulations were carried out after the approval of the ethics committee of the Cheikh Anta Diop University of Dakar (UCAD), at the laboratory of pharmaceutical physiology of the faculty of medicine, pharmacy and dentistry of the said university.

2.5. Statistical Analysis

The data obtained for the preventive test were analyzed using Prism version 5 software and then translated into contraction curves relative to the concentrations of Acetylcholine.

3. Results

In order to study the preventive effect of the studied plant extract on tracheal hyperreactivity, we chose acetylcholine as the contractile agent. First, we studied the contractile response of the trachea to acetylcholine administered cumulatively at doses of 10^{-5} mol/l to 4×10^{-5} mol/l without the tracheal ring being treated with the extract. In a second step, we incubated isolated rat tracheal rings with the hydroalcoholic extract of *Terminalia avicennioides* leaves at a concentration of 1 g/l and then studied the contractile response to acetylcholine administered cumulatively at the same previous doses. Our goal was to compare the contractile response to acetylcholine administered to the trachea with or without impregnation with the studied extract.

- **Effect of acetylcholine on the trachea**

The results, as shown in Figure 1, indicated that acetylcholine contracted the trachea and that the increase in its contractility depended on the dose of Ach added cumulatively.

- **Effects of the hydroalcoholic extract of *Terminalia avicennioides* leaves on the contractile response to acetylcholine**

The administration of the hydroalcoholic extract of *Terminalia avicennioides* leaves resulted in a modification of the contractile response to acetylcholine. Indeed, a significant attenuation of the contractile responses of the tracheal rings treated with the extract was observed compared to those that were not treated (control), as shown in Figure 2. At a certain dose, there was a sort of stabilization of the contractile responses to acetylcholine compared to the control, where an increasing contractility was observed with increasing doses of Ach added.

4. Discussion

During our work, we aimed to characterize the effects of a plant from the Senegalese pharmacopoeia on the contractile response of the isolated rat trachea. Indeed, many medicinal plants were commonly used in traditional therapy without scientific validation. Therefore, from isolated organ experiments, we studied the

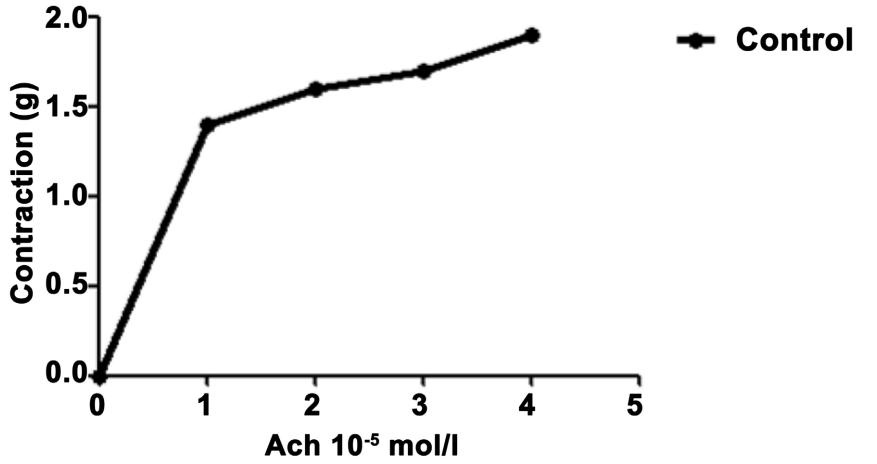

Figure 1. Effect of acetylcholine on the rat trachea.

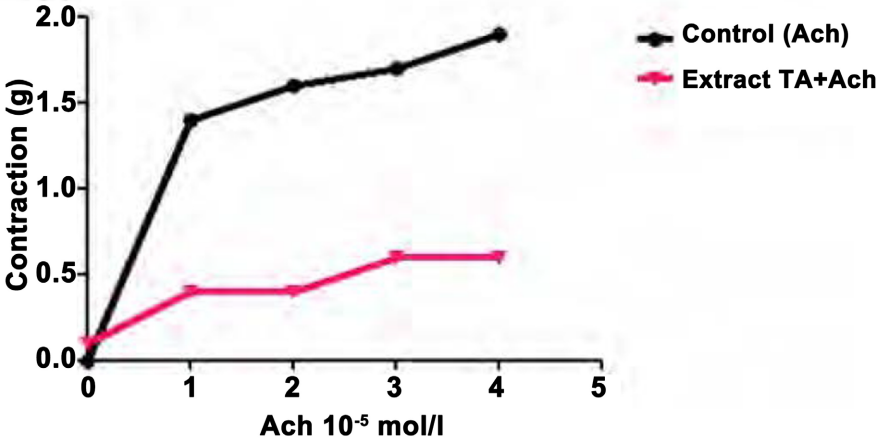

Figure 2. Effects of the hydroalcoholic extract of *Terminalia avicennioides* concentrated at 1 g/l on the contractile response to Ach at cumulative doses (10^{-5} to 4×10^{-5} mol/l) after a time of impregnation of the trachea with l'extracted for 30 min.

ability of the extract obtained from *Terminalia avicennioides* leaves to modulate the responses induced by acetylcholine at the level of the trachea.

During our work, we chose the male Wistar rat isolated trachea due to the clarity and stability of its motor responses. Indeed, previous studies had reported the use of guinea pig trachea as well as Wistar rat and mouse trachea [12] [13]. The choice of the trachea was also made due to the difficulties in manipulating the bronchi in the rat model, considering the isolated organ system we had. Indeed, the diameter of the rat's bronchi was very small and therefore constitutes additional sources of difficulty during mounting on isolated organ rods. It is therefore easier to work on the trachea, which was more accessible and had the same receptors and functional properties as the rest of the airways. Therefore, the results of tracheal motility could be extrapolated to bronchial motility without risk of error.

Graça *et al.* (2007) administered the extract on pre-contracted tracheas with agonists (carbachol, histamine, etc.) [14] [15] according to a curative protocol.

In other studies, the trachea was incubated with the extract, and then contractility was tested with sufficient doses of the contractile agonist, and the results were often very promising [13] [16]. This second preventive protocol was the one we applied in our study. In fact, previous work in our laboratory had already shown several limitations on the *in vitro* ability of extracts to induce tracheal relaxation when administered curatively. This is why we used the same approach as Diene [17], who used a preventive protocol by impregnating the tracheas before studying the contractile responses to acetylcholine. To do this, we impregnated the tracheal rings with the extract of the studied plant before inducing contractile responses with Ach.

The choice of Ach as a contractile agonist was guided by its direct involvement in the pathophysiology of respiratory diseases such as asthma. Indeed, Ach is the neurotransmitter of the parasympathetic system that is involved in the main bronchoconstrictive command. It is the most important neurological control system for tone, reactivity, and bronchial secretions. Thus, acetylcholine released by post-ganglionic cholinergic fibers activates M3 muscarinic receptors present on target cells, thus causing bronchoconstriction.

For the choice of working concentrations for the plant extract, to avoid any risk of toxicity and also not having experimental data on this plant, we worked with the minimum active concentration in our experimental conditions, of the order of 1 mg/ml. The results obtained during our work allowed us to demonstrate that the hydroalcoholic extract of *Terminalia avicennioides* leaves significantly reduced the contractile responses induced by acetylcholine. This result could be of interest in preventing tracheal or bronchial hyperreactivity observed in certain respiratory diseases, particularly asthma.

Recently, the team of Graça and that of Delarcina conducted studies respectively on Mikania laevigata and Cecropia glazioni [15]. The hydro-alcoholic extract of Mikania laevigata was tested *in vitro* on rat tracheal segments and a concentration-dependent relaxation of the trachea was demonstrated, not related to epithelium-derived factors. This relaxation was correlated with changes in calcium mobilization. This team demonstrated that the concentration of histamine necessary to induce bronchospasm was multiplied by five after administration of the hydro-alcoholic extract of Cecropia glazioni and by two if it was the purified fraction of the extract. The observed effects were blocked by treatment with propranolol. They then incubated tracheal segments in the purified fraction at high concentration and observed a reduction of 13% to 55% in the maximal response of the tracheal muscle to histamine. This suggests that the reduction in contractile response to acetylcholine induced by the hydroalcoholic extract of *Terminalia avicennioides* leaves could be related to a $\beta 2$ mimetic effect of the extract.

5. Limitations of the Study

The study used male Wistar rats aged between 12 and 14 weeks. While this pro-

vides valuable insights, it's essential to recognize that animal models may not fully represent human physiology. The results may not directly translate to clinical practice in humans.

The study focused on *Terminalia avicennioides* leaves. However, variations in plant growth conditions, harvesting methods, and extraction processes can lead to differences in bioactive compounds. Using extracts from different sources or parts of the plant could provide a more comprehensive understanding.

While the study suggests a $\beta 2$ mimetic activity, the exact mechanisms underlying the relaxing effect on tracheal smooth muscle remain unclear. Further research should explore specific pathways and receptors involved.

6. Conclusions

The results of our study have shown that the extract of *Terminalia avicennioides* leaves led to a significant attenuation of the contractile response induced by acetylcholine. Based on this result obtained after several tests of this plant, we can say that *Terminalia avicennioides* has a relaxing effect on the tracheal smooth muscle, and this effect could be related to a $\beta 2$ mimetic activity of the extract. This could play an important role in preventing bronchial hyperreactivity in certain respiratory diseases, particularly asthma.

This work should be further investigated to confirm and better appreciate the result obtained. Therefore, it would be interesting to characterize *in vitro* the mechanisms involved in the inhibitory effects on tracheal tone, to study on animal models of respiratory pathologies the ability of this plant to prevent asthma, and to identify the bioactive compounds present in its extract and the receptors on which they would act.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

References

[1] WHO (2023) Asthma [Internet]. https://www.who.int/news-room/fact-sheets/detail/asthma

[2] Dharmage, S.C., Perret, J.L. and Custovic, A. (2019) Epidemiology of Asthma in Children and Adults. *Frontiers in Pediatrics*, **7**, 246. https://doi.org/10.3389/fped.2019.00246

[3] Kwizera, R., Musaazi, J., Meya, D.B., Worodria, W., Bwanga, F., Kajumbula, H., *et al.* (2019) Burden of Fungal Asthma in Africa: A Systematic Review and Meta-Analysis. *PLOS ONE*, **14**, e0216568. https://doi.org/10.1371/journal.pone.0216568

[4] Refiloe, M., Kevin, M., Rebecca, N., Maia, L., Hellen, M., Graham, D., *et al.* (2022)

Asthma Care in Sub-Saharan Africa: Mind the Gap! *Journal of the Pan African Thoracic Society*, **3**, 59-62. https://doi.org/10.25259/JPATS_12_2022

[5] Skevaki, C., Ngocho, J.S., Amour, C., Schmid-Grendelmeier, P., Mmbaga, B.T. and Renz, H. (2021) Epidemiology and Management of Asthma and Atopic Dermatitis in Sub-Saharan Africa. *Journal of Allergy and Clinical Immunology*, **148**, 1378-1386. https://doi.org/10.1016/j.jaci.2021.10.019

[6] WHO (2023) World Health Organization - Regional Office for the Eastern Mediterranean. WHO EMRO|Maladies respiratoires chroniques|Thèmes de santé. http://www.emro.who.int/fr/health-topics/chronic-respiratory-diseases/

[7] Naghavi, M., Antony, C. and Brauer, M. (2023) Global Burden of Chronic Respiratory Diseases and Risk Factors, 1990-2019: An Update from the Global Burden of Disease Study 2019. *eClinicalMedicine*, **59**, 101936. https://www.healthdata.org/research-analysis/library/global-burden-chronic-respiratory-diseases-and-risk-factors-1990-2019 https://doi.org/10.1016/j.eclinm.2023.101936

[8] Dulin, N.O., Fernandes, D.J., Dowell, M., Bellam, S., McConville, J., Lakser, O., *et al.* (2003) What Evidence Implicates Airway Smooth Muscle in the Cause of BHR? *Clinical Reviews in Allergy & Immunology*, **24**, 73-84. https://doi.org/10.1385/CRIAI:24:1:73

[9] Belghitri, A. and Zemour, L. (2018) La place de la phytothérapie dans la prise en charge de l'asthme bronchique. *Revue des Maladies Respiratoires*, **35**, A92. https://doi.org/10.1016/j.rmr.2017.10.200

[10] Patrick, H., Honorine, N. and Nzabandora, C. (2015) Les plantes médicinales utilisées dans le traitement de l'asthme à l'ile d'Idjwi (Sud-Kivu, R.D. Congo). *International Journal of Innovation and Scientific Research*, **19**, 49-60.

[11] Mangambu Mokoso, J.D.D., Vasombolwa, K. and Félicien, B. (2008) Les plantes médicinales utilisées dans le traitement de l'asthme à Kisangani et ses environs (Province Orientale, R.D. Congo). *Annales des Sciences*, *Université Officielle de Bukavu*, **1**, 63-68.

[12] François-Bernard, M., *et al.* (1981) Asthmologie. Sandoz Editions, Paris, 301.

[13] Arulmozhi, D.K., Sridhar, N., Veeranjaneyulu, A. and Arora, S.K. (2006) Preliminary Mechanistic Studies on the Smooth Muscle Relaxant Effect of Hydroalcoholic Extract of *Curcuma caesia*. *Journal of Herbal Pharmacotherapy*, **6**, 117-124. https://doi.org/10.1080/J157v06n03_06

[14] Sánchez-Mendoza, M.E., Torres, G., Arrieta, J., Aguilar, A., Castillo-Henkel, C. and Navarrete, A. (2007) Mechanisms of Relaxant Action of a Crude Hexane Extract of *Gnaphalium liebmannii* in Guinea Pig Tracheal Smooth Muscle. *Journal of Ethnopharmacology*, **111**, 142-147. https://doi.org/10.1016/j.jep.2006.11.001

[15] Graça, C., Baggio, C.H., Freitas, C.S., Rattmann, Y.D., de Souza, L.M., Cipriani, T.R., *et al.* (2007) *In Vivo* Assessment of Safety and Mechanisms Underlying *in Vitro* Relaxation Induced by *Mikania laevigata* Schultz Bip. ex Baker in the Rat Trachea. *Journal of Ethnopharmacology*, **112**, 430-439. https://doi.org/10.1016/j.jep.2007.03.026

[16] Ghayur, M.N., Khan, H. and Gilani, A.H. (2007) Antispasmodic, Bronchodilator and Vasodilator Activities of (+)-Catechin, a Naturally Occurring Flavonoid. *Archives of Pharmacal Research*, **30**, 970-975. https://doi.org/10.1007/BF02993965

[17] Diene, D., *et al.* (2008) Caractérisation *in vitro* des effets d'extraits de plantes de la pharmacopée traditionnelle Sénégalaise sur la motricité trachéale. Thèse en pharmacie, Cheikh Anta Diop de Dakar. http://bibnum.ucad.sn/viewer.php?c=thm&d=thm%5f46709