Mangifera indica Stem Bark Effect on the Rat Trachea Contracted by Acetylcholine and Histamine

Amegnona Agbonon, Kodjo Aklikokou, and Messanvi Gbeassor
Centre de Recherche et de Formation sur les Plantes Médicinales (CERFOPLAM), Faculté des Sciences, Université de Lomé, Lomé, Togo

Abstract
Experiments were designed to determine the effect of Mangifera indica Linn stem bark aqueous extract on rat trachea previously incubated in presence of indomethacin, propranolol, and atropine or promethazine with histamine or acetylcholine as agonist. The strips of trachea were suspended for isometric tension recording at 37°C. M. indica aqueous extract at 2 mg/ml impair the contraction induced both by histamine and acetylcholine in all three experimental conditions. The contractile responses (% maximum effect) induced by histamine at 12 × 10⁻⁵ g/ml, in the presence of extract, were respectively 69.18 ± 3.05% (indomethacin), 74.18 ± 1.03% (indomethacin + propranolol), and 45.54 ± 1.7% (indomethacin + propranolol + atropine) versus 100 ± 5.15% for control. The extract also reduced the contraction induced by acetylcholine, but this inhibitory effect is lightly decreased when the tissues were contracted by acetylcholine after incubation in presence of indomethacin + propranolol. These experiments suggest that the aqueous extract of M. indica could block both the histaminic and muscarinic receptors on rat trachea; and the results corroborate with the traditional use of M. indica stem bark in the treatment of asthma.

Keywords: Acetylcholine, asthma, bronchoconstrictors, histamine, Mangifera indica, rat trachea.

Introduction
Mangifera indica Linn (Anacardiaceae), a common tree originally from India, is distributed in the tropical regions in the world. Known in Mina, the local language in Togo, as mangoti, different parts of M. indica are reported for many purposes. The leaves, traditionally used as an antimalarial, have been tested for their antimicrobial (De Souza et al., 1995) and antidiarrheal (Akendengué, 1992) effects. The stem bark of M. indica is used traditionally to treat female sterility and asthma disease. In previous work, we showed that the aqueous extract of stem bark relaxes the airway smooth muscle (Agbonon et al., 2002). Airway smooth muscle contraction, one of the characteristics of asthma disease, is induced mainly by histamine, a proinflammatory mediator, and a neurotransmitter as acetylcholine. On the airways, the degranulation of mast cells releases histamine, which provokes bronchospasm and explains airway hypersensitivity (Barnes et al., 1998). In addition to this direct action, histamine also elicits airway narrowing by neuronal mechanisms involving vagal reflex (Costello et al., 1999). Therefore, the aim of this investigation was to study the effect of the aqueous extract of M. indica on the rat isolated trachea in the presence of histamine and acetylcholine, the most potent bronchoconstrictors in airway narrowing, in different experimental conditions.

Materials and Methods
Plant material
The stem bark of M. indica was collected from the Lomé area in April 1999 and identified by Dr. Kofi Akpagana, Department of Botany, University of Lomé. A voucher specimen is deposited in the Herbarium of the department under reference No. 4949. The stem bark was dried at room temperature and powdered. The powder (300 g) was extracted with continuous agitation in distilled water (3 l) at 50°C for 30 min. The extraction yield of dried extract was approximately 8.26%. This aqueous extract

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Address correspondence to: Messanvi Gbeassor, Centre de Recherche et de Formation sur les Plantes Médicinales (CERFOPLAM), Université de Lomé, B.P. 1515 Lomé, Togo. E-mail: gbeassor@tg.refer.org

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was soluble in water, and the extract solution was prepared daily.

Animals and organ preparation

Wistar rats of either sex (130–150 g) were used. These animals, produced by the Department of Physiology/Pharmacology of University of Lomé, were kept under natural environmental conditions with a 12-h dark-light cycle and had free access to food and water.

These animals were sacrificed under ether anesthesia, and the trachea was quickly removed and transferred in cold Kreb’s solution. After removal of excess tissue, the strip of trachea was mounted in 30 ml UGO Basile Baths in Kreb’s solution and connected to the Universal Gould Force Transducer (Gould Instruments, Ballainvilliers, France). Changes in isometric force were recorded on a two-channel Thermal Writing Gould Recorder Series 8000s as described previously (Agbonon et al., 2002). The load applied to the tissue was 1 to 1.5 g. After these procedures, the preparation was allowed to equilibrate for 45 min in Kreb’s solution at 37°C gassed with air generator, and during this period, the preparation was washed three times.

Procedure

After equilibration, tissue was treated with the aqueous extract of *M. indica* at 2 mg/ml 10 min prior cumulative doses of histamine (10^-5 to 12 × 10^-4 g/ml) or acetylcholine (10^-8 to 5 × 10^-4 g/ml). Previously, cumulative doses of histamine (n = 11) or acetylcholine (n = 9) were tested only on the tissues as controls.

In another set of experiments, and for studying the antihistaminic effect of *M. indica*, the strip of trachea was incubated 20 min before the extract application in three different conditions as follows: indomethacin (5 μg/ml), indomethacin + propranolol (0.6 μg/ml), or indomethacin + propranolol + atropine (5 μg/ml) as described previously (Boskabady & Shaikhi, 2000). Ten minutes after extract application, cumulative doses of histamine, as indicated previously, were applied. Each organ was used once only for each condition with n = 5 rats.

The same procedure was used to evaluate the effect of *M. indica* on muscarinic receptors on rat tracheal smooth muscle. But for this purpose, promethazine (5 μg/ml) was used to serve as substitute for atropine in the third experimental condition.

Statistical analysis

Data are expressed as mean ± SEM, and statistical analysis was performed by ANOVA followed by Fisher least significance difference test (LSD), using Systat 5.0. Results are significant if the probability p < 0.05.

Results

Cumulative doses of histamine (10^-5 to 12 × 10^-4 g/ml) induced the contraction of the strip of trachea in a dose-dependent manner. The tissues developed a sustained tension for many minutes when the last dose of histamine (12 × 10^-4 g/ml) was applied. Tension returned to baseline after the tissue was washed (data not shown). The aqueous extract of *M. indica* stem bark at 2 mg/ml, applied 10 min prior to the cumulative doses of histamine, reduced the contraction induced by histamine. The contractile responses (% maximum effect) induced by histamine at 9 × 10^-4 and 12 × 10^-4 g/ml were, respectively, 8.03 ± 1.7% and 60.17 ± 4.46% in the presence of extract, versus 59.72 ± 7.58% and 100 ± 5.15% for control (Fig. 1). After tissue incubation in the presence of indomethacin, propranolol, or atropine, the contractile responses induced by histamine at 12 × 10^-4 g/ml, in the presence of extract were, respectively, 69.18 ± 3.05%, 74.18 ± 1.03%, and 45.54 ± 1.7% (Figs. 1, 2, and 3). These results show that in all three experimental conditions, the inhibitory effect of the extract is not abolished. Moreover, the cumulative concentration-response curves of histamine produced in the presence of extract in all three experimental conditions show a clear rightward and downward shift compared to histamine-response curve released without extract (Figs. 1, 2, and 3). From these curves, the EC_{50} of histamine obtained in the presence of extract in all three experimental conditions was higher than those for control (Table 1).

Acetylcholine (10^-8 to 5 × 10^-4 g/ml) contracted the strip of trachea as histamine in a dose-dependent manner. This contraction was sustained and maintained as long as the tissue was not washed. The high tension is induced by 2.5 × 10^-4 g/ml of acetylcholine because there

![Figure 1](image-url)
is transient decrease of the tension after application of $5 \times 10^{-4}$ g/ml of acetylcholine (data not shown). The extract applied in the same manner as described previously reduced the tension induced by acetylcholine. The contractile responses induced by acetylcholine at $2.5 \times 10^{-4}$ g/ml in the presence of the extract, after incubation in the presence of indomethacin + propranolol, is $90.91 \pm 9.27\%$ versus control acetylcholine, which is 100\%. These results show that the inhibitory effect of *M. indica* extract on the trachea is impaired in the presence of indomethacin + propranolol. However, the cumulative concentration-response curves of acetylcholine released in the presence of extract in all three experimental conditions (indomethacin, propranolol, and promethazine) showed a clear rightward and downward shift compared to acetylcholine-response curve released without extract (Figs. 4, 5, and 6). The EC$_{50}$ of acetylcholine obtained from these curve is $3.98 \times 10^{-6}$ g/ml in the presence of extract only. The EC$_{50}$ in the presence of extract in all three experimental conditions was $15.84 \times 10^{-6}$ g/ml in presence of indomethacin + *M. indica* and $3.16 \times 10^{-6}$ g/ml in presence of indomethacin + propranolol + *M. indica*. All these values are higher than control value ($10^{-6}$ g/ml) (Table 2).

**Discussion**

We have shown in the current study that the aqueous extract of *M. indica*, applied 10 min prior to agonists, has inhibited the contraction induced by the cumulative doses of histamine or acetylcholine; the extract reduced the effect of histamine and acetylcholine after incubation

![Figure 2. Cumulative log concentration-response curves of histamine on isolated trachea of rat in presence of extract on the preparations incubated with indomethacin + propranolol. Each value is mean ± SEM with n = 5 (extract) and n = 11 (control histamine); *p < 0.01 vs. control histamine.](image)

![Figure 3. Cumulative log concentration-response curves of histamine on isolated trachea of rat in presence of extract on the preparations incubated with indomethacin + propranolol + atropine. Each value is mean ± SEM with n = 5 (extract) and n = 11 (control histamine); *p < 0.01 vs. control histamine.](image)

![Figure 4. Cumulative log concentration-response curves of acetylcholine on isolated trachea of rat in presence of extract on the preparations incubated with indomethacin. Data are mean ± SEM with n = 5 (extract and control indomethacin) and n = 9 (control acetylcholine); *p < 0.05 vs. control indomethacin; *p < 0.05 and **p < 0.01 vs. control acetylcholine.](image)
of the tissues in presence of indomethacin, propranolol, and atropine or promethazine.

Indomethacin inhibits the synthesis of prostaglandins like PGF<sub>2</sub>, which is an endogenous bronchodilator. Moreover, incubation of the tissues in the presence of indomethacin enhanced the synthesis of leukotrienes, which are frank bronchoconstrictors (Underwood et al., 1997). Propranolol, a β-adrenergic receptor inhibitor, causes bronchoconstriction (Fujimura et al., 1999). Indeed, β2-adrenergic receptor agonist induced bronchodilatation through adenyl-cyclase stimulation. For this aim, incubation of the tissues in presence of propranolol suppresses β2-agonist relaxation pathway. Atropine is a nonspecific muscarinic receptor antagonist, and promethazine is a histaminic receptor antagonist. The extract relaxes the tissue contraction induced both by histamine and acetylcholine in all these three experimental conditions. These results suggest that the extract has antihistaminic and antimuscarinic effects on rat trachea. In addition, these results suggest that the extract could inhibit leukotrienes synthesis, because there are significant differences (p < 0.05) between histamine-response curve in the presence of indomethacin with extract and without extract. These major findings provide a strong rationale for the traditional use of <i>M. indica</i> stem bark in the treatment of asthma and were supported by a previous study in which <i>M. indica</i> extract impaired the contraction of the trachea induced by acetylcholine.

In conclusion, the aqueous extract of <i>M. indica</i> inhibits the contraction of the trachea induced by histamine and acetylcholine. This inhibition is maintained in our three experimental conditions. These results show that the extract could have direct actions on histaminic and muscarinic receptors. Inhibition of the contraction of the trachea by the extract corroborates with the traditional use of <i>M. indica</i> in the treatment of asthma; but further investigations are needed to evaluate the effect of extract on other parameters of asthma disease.

References


M. indica effect on contracted rat trachea