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Effects of Some Malian Medicinal Plants on the Respiratory Tract of Guinea-pigs

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Abstract

Crossopteryx febrifuga, *Pteleopsis suberosa* and *Entada africana* are used in Mali traditional medicine for fever and various respiratory diseases. We have investigated the effects of these three drugs in the form of a decoction on the respiratory tract using different experimental models.

On citric acid-induced cough in guinea-pigs, the three drugs significantly decreased the number of coughs at the doses of 250 ($P < 0.01$), 500 ($P < 0.05$; $P < 0.01$) and 1000 ($P < 0.01$) mg kg^{-1}. The percent inhibition was respectively 62·86, 69·03 and 77·44% for *C. febrifuga*, 57·80, 53·90 and 61·40% for *E. africana*, and 37·13, 42·44 and 73·72% for *P. suberosa*. Codeine phosphate (10 mg kg^{-1}) used as reference drug showed an inhibition of 76·32%.

E. africana (1000 mg kg^{-1}) reduced (65% inhibition) significantly ($P < 0.05$) bronchoconstriction induced by histamine (99·25% and 34·00% for control and extract, respectively). Furthermore, *E. africana* (1000 mg kg^{-1}) provoked a bronchodilatation response when administered under basal conditions.

On antigen-induced bronchospasm, *C. febrifuga* protected (54% inhibition) sensitized guinea-pigs with a pulmonary ventilation pressure (PVP) of 24·87% (control value $< 55.00\%$). *P. suberosa* was inactive in both experimental models. The reference drug, disodium cromoglycate (10 mg kg^{-1}, i.v.) protected significantly ($P < 0.05$) with a PVP of 12·00% (78% of inhibition).

This study confirmed the traditional use of these plants in the treatment of cough and other respiratory disorders.

In developing countries, the use of traditional remedies is a common practice and a large number of plants is used. In Africa, the most accessible health care provider is the traditional medical practitioner, who has in his possession a large quantity of effective herbal remedies. Ethnomedicine provides a source of information about these plants, the investigation of which reveals a reservoir of pharmacologically active substances for treatment of various diseases. Diverse plants are used in Malian traditional medicine for fever and respiratory tract diseases, bronchitis, colds, cough, bronchopneumonia, whooping-cough, inflammation of oropharynx and hiccough (Kerharo & Adam 1974; Oliver-Bever 1983). The Department of Traditional Medicine of Mali produced an antitussive syrup, "Improved Folk Prescription" (Balembo), prepared with the fruits of *Crossopteryx febrifuga*.

In this study, the antitussive activity of *C. febrifuga* fruit (Rubiaceae), *Pteleopsis suberosa* stem bark (Combretaceae) and *Entada africana* root (Mimosaceae) has been evaluated using the classical model of citric acid-evoked cough in guinea-pigs. Their effects on bronchial resistance in basal conditions, on histamine-induced bronchoconstriction or antigen-induced bronchospasm in guinea-pigs were also studied.

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Materials and Methods

Plant material

Plant material was collected in July 1996 in the belt of Bamako (Mali) and identified by the Traditional Medicine Department. The specimens are preserved in the herbarium for future reference.

Preparation of extracts

The extracts were prepared by boiling 100 g powdered dried plant with 1000 mL water for 30 min. After filtration (Whatman no. 4 filter paper), each extract was lyophilized. The yields obtained were 10·94% for *Crossopteryx*, 7·40% for *Entada* and 21·36% for *Pteleopsis*. For administration, the residue was dissolved in water (10 mL kg^{-1}).

Citric acid spray-evoked cough in guinea-pigs

Male guinea-pigs (Charles River; Calco-Italia), 550–700 g, were housed under standard laboratory conditions with free access to food and water. According to the methods reported by Ucelay et al (1991), animals were placed individually in a transparent box (20 × 14 × 12 cm) and exposed for 5 min to an aerosol of 10% w/v citric acid in water. The aerosol was produced by air compressed at a flow rate of 0·16 L s^{-1} and a pressure of 0·5 bar. The aerodynamic mass diameter of the particles generated by the nebulizer that contained 5 mL citric acid, was from 0·6 to 15 μm. During exposure, the animals were observed and the time to the onset of the first cough, the total number of coughs during the 5 min of exposure and during the succeeding 5 min were recorded. The day before the exposure test each animal had been tested to obtain the control response. Only animals producing 10–25 coughs during a 5-min period in the preliminary selection were used.

The next day, animals were fasted for 24 h, but water was freely available. The animals were divided into 10 groups of five. Groups 1, 2 and 3 were treated with *C. febrifuga* (250, 500 or 1000 mg kg^{-1}, p.o.), groups 4, 5 and 6 with *P. suberosa* (250, 500 or 1000 mg kg^{-1}, p.o.) and groups 7, 8 and 9 with *E. africana* (250, 500 or 1000 mg kg^{-1}, p.o.). The 250-mg dose was comparable to the therapeutic dosage used in Mali of 15 g/day (250 mg kg^{-1} of powdered dried plant for an average weight of 60 kg). The other doses (500 and 1000 mg) were employed as evidence of the dose-dependent activity of the extracts. The doses of extracts administered to each group corresponded to mg dried plant. The reference drug, codeine phosphate (10 mg kg^{-1}) was administered to group 10. One hour after treatment, all the animals were exposed to citric acid aerosol.

The antitussive activity was calculated as the percentage inhibition of the number of coughs between the second and the first exposure.

Histamine-induced bronchoconstriction in guinea-pigs

Male guinea-pigs, 300–418 g, were housed under standard laboratory conditions with free access to food and water.

The effects of the *C. febrifuga*, *P. suberosa* and *E. africana* extracts on bronchial resistance were studied by a modification of the method of Konzett & Rossler (1940). The animals, fasted for 24 h with water freely available, were divided into four groups of five. Group 1 served as a control and were administered the vehicle (water) (10 mL kg^{-1}, p.o.). Groups 2, 3 and 4 were treated with *C. febrifuga*, *P. suberosa* and *E. africana* extracts (1000 mg kg^{-1} of dried plant, p.o.), respectively. One hour later, all animals were anaesthetized with ethyl urethane (1·25 g kg^{-1}, i.p.), placed in the supine position and ventilated artificially at a frequency of 50 breaths min^{-1} through a tracheal cannula; respiratory volume was adjusted to 10 mL/breath. The ventilation pressure in the circuit (pump–guinea-pig) was measured with a bronchospasm transducer 7020 connected to a recorder Gemini 7070 (both items, Ugo Basile, Camerio, Italy). The jugular vein was cannulated in order to administer 20 μg kg^{-1} histamine. Histamine-induced bronchoconstriction was assessed with or without drug administration. Changes in pulmonary ventilation pressure (PVP) were expressed as a % of the basal PVP and the % inhibition was based on the comparison of the test and control % PVP values.

Antigen-induced bronchospasm in guinea-pigs

Male guinea-pigs, 300–418 g, were divided into five groups of five. All animals were sensitized first with a nebulized Oleaceae allergen (phenolic extract 0·4% SARM-ROME) by two successive inhalations (50 μL each) in order to obtain the maximal bronchospasm upon challenge (Quattrone et al 1990). After 48 h, the animals fasted for 24 h, with water freely available, were anaesthetized with ethyl urethane (1·25 g kg^{-1}, i.p.), placed under assisted respiration as described above and administered with the Oleaceae allergen (100 μL) by intratracheal instillation. Group 1 served as a control and were administered the vehicle (water, 10 mL kg^{-1}, p.o.). Groups 2, 3 and 4 were treated

with *C. febrifuga*, *P. suberosa* and *E. africana* extracts (1000 mg kg^{-1} of dried plant, p.o.), respectively, 1 h before intratracheal instillation of the Oleaceae allergen. The reference drug, disodium cromoglycate (10 mg kg^{-1}, i.v.) was administered to group 5, 15 min before the allergen.

Antigen-induced bronchospasm was assessed with or without drug administration. Changes in PVP and % inhibition were calculated as described above.

Statistical analysis

All data are expressed as mean ± s.d. Results were analysed using Student's *t*-test for paired parametric data and differences were considered significant for $P < 0{\cdot}05$.

Results

Exposure of guinea-pigs to nebulized 10% citric acid consistently induced coughing. The number of coughs and the latency time of the first cough after citric acid exposure were quite variable, however, animals were used as their own controls. *C. febrifuga*, *E. africana* and *P. suberosa* decoctions, at increasing doses, significantly inhibited the number of coughs and increased the latency time of the first cough in guinea-pigs (Tables 1, 2 and 3, respectively).

The effects of the three drugs on bronchial resistance in basal conditions were evaluated. Only *E. africana* (1000 mg kg^{-1}) produced a bronchodilatator effect with a 24·20% drop of PVP with respect to basal values (data not shown).

Table 1. The antitussive effects of *Crossopteryx febrifuga* extract and codeine on citric acid-evoked cough in guinea-pig.

Treatments	Delay (s)		Number of coughs		Inhibition (%)
	Before treatment	After treatment	Before treatment	After treatment	
Crossopteryx (250 mg kg^{-1})	72·0 ± 26·8	108·0 ± 26·8	21·2 ± 4·4	7·8 ± 1·5**	62·9
Crossopteryx (500 mg kg^{-1})	84·0 ± 32·9	168·0 ± 50·2	20·6 ± 6·8	6·8 ± 3·9**	69·0
Crossopteryx (1000 mg kg^{-1})	72·0 ± 26·8	144·0 ± 53·7	17·2 ± 4·9	3·8 ± 1·3**	77·4
Codeine (10 mg kg^{-1})	96·0 ± 32·9	168·0 ± 50·2	15·2 ± 3·5	3·6 ± 0·9**	76·3

Data are expressed as mean ± s.d., n = 5. **$P < 0{\cdot}01$ compared with control. Doses of *Crossopteryx febrifuga* are expressed as the amount of dried plant.

Table 2. The antitussive effects of *Entada africana* extract and codeine on citric acid-evoked cough in guinea-pig.

Treatments	Delay (s)		Number of coughs		Inhibition (%)
	Before treatment	After treatment	Before treatment	After treatment	
Entada (250 mg kg^{-1})	144·0 ± 32·9	204·0 ± 32·9	13·2 ± 3·1	5·4 ± 1·3**	57·8
Entada (500 mg kg^{-1})	96·0 ± 32·9	156·0 ± 32·9	17·4 ± 7·1	7·2 ± 0·8*	53·9
Entada (1000 mg kg^{-1})	132·0 ± 26·8	168·0 ± 26·8	14·2 ± 2·2	3·2 ± 0·8**	61·4
Codeine (10 mg kg^{-1})	96·0 ± 32·9	168·0 ± 50·2	15·2 ± 3·5	3·6 ± 0·9**	76·3

Data are expressed as mean ± s.d., n = 5. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared with control. Doses of *Entada africana* are expressed as the amount of dried plant.

Table 3. The antitussive effects of *Pteleopsis suberosa* extract and codeine on citric acid-evoked cough in guinea-pig.

Treatments	Delay (s)		Number of coughs		Inhibition (%)
	Before treatment	After treatment	Before treatment	After treatment	
Pteleopsis (250 mg kg^{-1})	96·0 ± 32·9	120·0 ± 0·0	18·4 ± 1·3	11·6 ± 1·7**	37·1
Pteleopsis (500 mg kg^{-1})	108·0 ± 26·8	132·0 ± 65·7	15·0 ± 2·8	9·80 ± 1·9**	42·4
Pteleopsis (1000 mg kg^{-1})	144·0 ± 32·8	168·0 ± 26·8	14·4 ± 2·2	3·20 ± 0·8**	73·7
Codeine (10 mg kg^{-1})	96·0 ± 32·9	168·0 ± 50·2	15·2 ± 3·5	3·60 ± 0·9**	76·3

Data are expressed as mean ± s.d., n = 5. **$P < 0{\cdot}01$ compared with control. Doses of *Pteleopsis suberosa* are expressed as the amount of dried plant.

The effects of the drugs on histamine-induced bronchoconstriction and on antigen-induced bronchospasm in guinea-pigs are shown in Tables 4 and 5, respectively. In the histamine-induced bronchoconstriction experimental model, PVP was recorded, and the results of the drug-treated groups were compared with controls. Only *E. africana* was able to reduce significantly ($P < 0{\cdot}05$) the bronchoconstriction induced by histamine (PVP 34·00% and 99·25% for extract and control, respectively; 65% of inhibition; Table 4).

Results in Table 5 show that *C. febrifuga* extract protected significantly ($P < 0{\cdot}05$) against antigen-induced bronchospasm with a mean PVP of 24·87% compared with 55·00% for the controls (54% of inhibition). *P. suberosa* was inactive in both experimental models.

Disodium cromoglycate, widely used in the prophylaxis of bronchial asthma, protected significantly ($P < 0{\cdot}05$) against antigen-induced bronchospasm with a mean PVP of 12·00% (78% of inhibition).

Table 4. Effect of *Crossopteryx febrifuga*, *Entada africana* and *Pteleopsis suberosa* extracts on histamine-induced bronchoconstriction in guinea-pigs.

Treatments	Dose (mg kg^{-1})	Pulmonary ventilation pressure (PVP, %)	Inhibition (%)
Control	–	99·25 ± 21·92	–
Crossopteryx	1000	99·19 ± 20·89	–
Entada	1000	34·00 ± 9·31*	65
Pteleopsis	1000	99·21 ± 22·88	–

Changes in PVP are expressed as a % of the basal PVP. % inhibition is based on the comparison between the test and control % PVP values. Data are expressed as mean ± s.d., n = 5. *$P < 0{\cdot}05$ compared with control. Doses are expressed as the amount of dried plant.

Table 5. Effect of *Crossopteryx febrifuga*, *Entada africana*, *Pteleopsis suberosa* extracts and disodium cromoglycate on antigen-induced bronchospasm in guinea-pigs.

Treatments	Dose (mg kg^{-1})	Pulmonary ventilation pressure (PVP, %)	Inhibition (%)
Control	–	55·00 ± 10·60	–
Crossopteryx febrifuga	1000	24·87 ± 4·11*	54
Entada africana	1000	55·00 ± 9·25	–
Pteleopsis suberosa	1000	55·00 ± 11·30	–
Disodium cromoglycate	10	12·00 ± 1·15*	78

Changes in PVP are expressed as a % of the basal PVP. % inhibition is based on the comparison between the test and control % PVP values. Data are expressed as mean ± s.d., n = 5. *$P < 0{\cdot}05$ compared with control. Doses are expressed as the amount of dried plant.

Discussion

Inhalation of an aqueous solution of citric acid is known to cause airway chemoreceptor irritation in man and in guinea-pig, and this response appears to involve sensory mechanisms linked to the cough reflex (Kase 1968; Turner 1968). It has been demonstrated that citric acid-induced cough by stimulating sensitive sensory nerves and bronchoconstriction induced by citric acid in guinea-pigs involves a muscarinic mechanism (Allott et al 1980; Forsberg & Karlsson 1986; Forsberg et al 1988).

In this study, *C. febrifuga*, *P. suberosa* and *E. africana* extracts all inhibited citric acid-induced cough. It was found that *C. febrifuga* and *P. suberosa* extracts, administered at the highest dose, caused a significant inhibition of cough similar to the standard drug codeine.

Histamine-induced bronchoconstriction was also attenuated by *E. africana* treatment; the bronchodilator effect of the extract in basal conditions can partially explain the antihistaminic effects.

Antigen-induced bronchospasm in sensitized guinea-pigs is a widely used model of experimental asthma and the response is mediated by a variety of agents such as histamine, bronchoconstrictor prostaglandins, leukotrienes and platelet-activating factor among others (Tavares-Murta et al 1993). This study showed that oral administration of *Crossopteryx* extract did not affect the bronchospastic action of exogenous histamine, but it protected against antigen-induced bronchospasm. It is possible that the protective effect of the *Crossopteryx* extract could be due to its action on other pharmacological mediators implicated in allergic reactions.

Previous studies have reported that *C. febrifuga* contains alkaloids, triterpenoids (Oliver-Bever 1986), saponins (Gariboldi et al 1990) and flavonoids (Tomàs-Barberan & Hostettmann 1988). Many studies have reported the anti-allergic properties of flavonoids and their derivatives like disodium cromoglycate, a chromone known for its clinical use in the therapy of asthma (Gábor 1986). The presence of flavonoids in *C. febrifuga* extract may partially explain its effect against antigen-induced bronchospasm.

Phytochemical analysis of *P. suberosa* showed a high content of tannins (27%) and steroids, triterpenoid glycosides and saponins (De Pasquale et al 1995). It should also be noted that *C. febrifuga* and *P. suberosa* are active against microorganisms involved in respiratory infections (Sanogo et al 1998).

Our preliminary phytochemical analysis on *E. africana* extract has shown the presence of triterpenoids, saponins and tannins. The interactions

between these various compounds present in *E. africana* extract may produce antitussive and bronchodilatatory effects via different mechanisms.

In conclusion, the antitussive activity of the three plants and the beneficial effect of *C. febrifuga* against antigen-induced bronchospasm and *E. africana* against histamine-induced bronchoconstriction, may justify the popular use of these plants in cough associated to other respiratory diseases.

References

Allott, C. P., Evans, D. P., Marshall, P. W. (1980) A model of irritant-induced bronchoconstriction in the spontaneously breathing guinea-pig. Br. J. Pharmacol. 71: 165–168

De Pasquale, R., Germanò, M. P., Sanogo, R., Keita, A., Iauk, L. (1995) The antiulcer activity of *Pteleopsis suberosa*. J. Ethnopharmacol. 47: 55–58

Forsberg, K., Karlsson, J. A. (1986) Cough induced by stimulation of capsaicin-sensitive sensory neurons in conscious guinea-pig. Acta Physiol. Scand. 1: 319–320

Forsberg, K., Karlsson, J. A., Theodorsson, E., Lundberg, J. M., Persson, C. G. A. (1988) Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in the guinea-pig. Pulmonary Pharmacol. 1: 33–39

Gábor, M. (1986) Anti-inflammatory and anti-allergic properties of flavonoids. In: Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological, and Structure Activity Relationship. Alan R. Liss, Inc., New York, pp 471–480

Gariboldi, P., Verotta, L., Gabetta, B. (1990) Saponins from *Crossopteryx febrifuga*. Phytochemistry 29: 2629–2635

Kase, Y. (1968) In: Burger, A. (ed.) Selected Pharmacological Testing Methods. M. Dekker Inc., New York, p. 363

Kerharo, J., Adam, J. G. (1974) Pharmacopée Sénégalaise Traditionnelle: Plantes Médicinales et Toxiques. Editions Vigot-frères Paris, pp 353–356

Konzett, H., Rossler, R. (1940) Versuchsanordnung zu Untersuchungen an der Bronchialmuskur. Arch. Exp. Pathol. Pharmakol. 19: 71–74

Oliver-Bever (1983) Medicinal plants in tropical West Africa III. Anti-infection therapy with higher plants. J. Ethnopharmacol. 9: 1–80

Oliver-Bever (1986) Anti-infective activity of higher plants. In: Medicinal Plants in West Africa. Cambridge University Press, Cambridge, p. 165

Quattrone, G., Occhiuto, F., Circosta, C., Aliberti, S., Gregorio, A., Ando', F., Girbino, G. (1990) Modello di broncospasmo indotto con diversi estratti allergenici nella cavia anestetizzata. Accademia Peloritana dei Percolanti 77: 213–218

Sanogo, R., Crisafi, G., Germanò, M. P., De Pasquale, R., Bisignano, G. (1998) Evaluation of Malian traditional medicines: screening for antimicrobial activity. Phytotherapy Res. 12: S154–S156

Tavares-Murta, B. M., Lefort, J., Cunha, F. Q., Ferreira, S. H., Vargaftig, B. B. (1993) Interference of a neutrophil recruitment inhibitory factor upon the accumulation of inflammatory cells and airway hyperreactivity in sensitized guinea-pigs after intranasal antigen challenge. Br. J. Pharmacol. 108: 538–543

Tomàs-Barberan, F. A., Hostettmann, K. (1988) A cytotoxic triterpenoid and flavonoids from *Crossopteryx febrifuga*. Planta Med. 54: 266–267

Turner, R. A. (1968) Screening Methods in Pharmacology. Academic Press, New York, p. 218

Ucelay, M., Labeaga, L., Orjales, A., Zubiaur, L., Quintana, A. (1991) Evaluation of bronchospasmolytic, antiallergic, anti-inflammatory, mucolytic and antitussive activities of decasilate in experimental models. Arzneim. Forsch. 41: 528–532