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Antibronchoconstrictor Effects of *Securidaca Longipedunculata* (Fresen.) Root Bark Methanolic Extract in Guinea-pigs

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Summary. **This study was designed to examine the antibronchoconstrictor effects of *Securidaca longipedunculata* (Fresen.) root bark methanolic extract (MESL) in guinea-pigs. The plant extract relaxed spasmogen-(acetylcholine-, histamine-, serotonin-, and potassium-) induced contractions of the guinea-pig isolated tracheal muscle prepations in a concentration-related manner. The plant extract also protected guinea-pigs against histamine aerosol-induced bronchospasm *in vivo*. Neither the relaxant effects of the plant extract on spasmogen-evoked contractions of the guinea-pig isolated tracheal muscle preparations, nor its protective effects on histamine aerosol-induced bronchospasm in conscious guinea-pigs *in vivo* was blocked by propranolol, which blocked both the relaxant and protective effects of isoprenaline in the mammalian experimental model used. Although the exact mechanism of the bronchodilator effect of the plant extract remains speculative, it is unlikely that the plant extract stimulates β_2-adrenoceptors on the bronchial smooth muscle to produce its bronchodilation. However, it is thought that the antibronchoconstrictor effect of the plant extract is probably largely due to its non-specific spasmolytic activity, through which mechanism it acts to antagonise the post-junctional, spasmogenic and stimulant effects of acetylcholine, histamine, and other spasmogens on the bronchial smooth muscle. Furthermore, since saponins and sapogenins are known to possess non-specific spasmolytic properties against a wide range of spasmogens, it is thought that the bronchodilator activity of the plant extract may be attributed largely to presenegenin, a triterpenoid sapogenin present in the plant extract.**

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Key words—*Securidaca longipedunculata*, root bark methanolic extract, guinea-pig trcheal chain muscle preparations, spasmogen-induced contractions, histamine aerosol-induced bronchospasm, bronchodilator action.

INTRODUCTION

The ethnobotanical survey of African medicinal plants has revealed that more than one thousand plants from diverse families are used medicinally on the continent as folk remedies in the management/treatment of bronchial asthma. Asthma is an inflammatory condition of the respiratory system with widespread constriction and narrowing of the bronchial airways, which changes in severity over short periods of time, and leads to cough, wheezing, and difficulty in breathing. Bronchial asthma may be precipitated by exposure to one or more of a wide range of stimuli, including allergens, drugs, exertion, infection, and air pollution. Orthodox treatment of asthma involves the use of bronchodilators, with or without corticosteroids. In many parts of Africa, various morphological parts of *Securidaca longipedunculata* (Fresen.) (family: Polygalaceae) are used in folklore medicine to treat or manage a plethora of human ailments, including bronchial asthma. In South Africa, as in Nigeria, the roots of *S. longipedunculata* are used for coughs and chest complaints, respiratory problems, rheumatism, toothache, and headaches (Watt and Breyer-Brandwijk, 1962). The stem bark of the plant is an ingredient of arrow poisons, and the plant is known as "an ordeal poison" (Neuwinger, 1994). Because of the wide applications of *S. longipedunculata* in African folklore

medicine, the present study was undertaken to examine the antibronchoconstrictor effects of *S. longipedunculata* root bark methanolic extract *in vitro* and *in vivo* in guinea-pigs.

MATERIALS AND METHODS

Plant materials

Fresh root barks of *Securidaca longipedunculata* (Fresen.) were collected from Pietersburg (in Northern Province of South Africa) between November 1998, and February 1999. The root barks were identified to be those of *Securidaca longipedunculata* (Fresen.) by the staff of the Natal Provincial Herbarium in Durban, South Africa, as well as the staff of the Botany Department of the University of Durban-Westville, Durban, South Africa (where a voucher specimen of the plant has been deposited). Two kilogrammes (2 kg.) of sun-dried, brownish root barks of the plant were cut into small pieces and pulverised. Phytochemical processes of 99.5% methanol extraction of the powdered root barks were carried out as described in detail earlier by Odebiyi and Sofowora (1978). The resulting dark-brown methanol extract was concentrated *in vacuo* and freeze-dried. The extract residue (125 g.) was then refrigerated for subsequent pharmacological testing. Aliquot portions of the plant extract residue were weighed out and dissolved in distilled water on each day of our experiment.

Animal materials

Adult, Dunkin-Hartley guinea-pigs (*Cavia porcellus*) of both sexes weighing 300-350 g. were used. The antibronchoconstrictor effects of *S. longipedunculata* root bark methanolic extract were examined *in vitro* and *in vivo*.

EXPERIMENTALS

In vitro experiments

Guinea-pig isolated tracheal chain muscle preparations

The method used for these preparations was adopted from those described by Foster (1960) and Ojewole (1976). Each guinea-pig was killed by applying a sharp blow to the back of its head and then quickly bled out. The entire trachea of the animal was removed and cut into 5-7 approximately equal rings. Each ring was cut open through the cartilage, and 3-4 of such open rings were tied together to form a chain. The tracheal chain muscle preparation was suspended in a 10-ml organ bath containing Krebs-Henseleit physiological solution (of composition, in g/litre: NaCl, 6.92; KCl, 0.34; NaH_2PO_4, 0.15; $NaHCO_3$, 2.1; $MgCl_2$, 0.11; $CaCl_2$, 0.26; and glucose, 1.00) maintained at 32±1℃ (pH adjusted to 7.4), and continuously aerated with carbogen (i.e., 95% O_2 +5% CO_2 gas mixture) under an applied resting tension of 1 g. Each preparation was allowed to equilibrate for 45-60 min. (during which time the bathing physiological solution was changed every 15 min.) before it was challenged with drugs. The tracheal chain muscle preparation was contracted with sequential exogenous addition of either acetylcholine (1 μg/ml); histamine (1.5 μg/ml); 5-hydroxytryptamine (0.6 μg/ml); or potassium (K^+, 30 mM) to the bath fluid. The maximal muscle tension developed by the spasmogens used was similar, and approximately equal to 1.5 g. The relaxant effects of *S. longipedunculata* root bark methanolic extract (MESL) on the muscle tension developed by the spasmogens (agonists) were examined by cumulative additions of graded concentrations of the plant extract to the bath fluid when the maximal contractile effects of the spasmogens had been essentially obtained. In all cases, after a maximal relaxation to a relaxant agent had been achieved, the muscle preparation was washed out 3-5 times with fresh Krebs-Henseleit physiological solution, and then left to recover for 20-30 min. before it was contracted again with any of the standard spasmogens. Changes in tension developed by the muscle (contractions and relaxations) were recorded isometrically by means of Ugo Basile force-displacement transducers and pen-writing "Gemini" microdynamometers (model 7070). In order to make allowance for changes in muscle sensitivity, two preparations from the same animal were always set up (one used as "control" and the other one "drug-treated"). The control muscle preparations were always treated with distilled water (0.1-0.5 ml) only. In these *in vitro* experiments, papaverine (50-800 μg/ml) and isoprenaline (0.1-1.0 μg/ml) were used as reference tracheal chain muscle relaxants for comparison.

In vivo experiments

Histamine aerosol-induced bronchospasm in conscious guinea-pigs

The method used in this study was essentially that described in detail by Ojewole (1976). Ten control adult guinea-pigs and ten other adult guinea-pigs for each test compound were separately placed in suitable chambers. Control group guinea-pigs were

sprayed with distilled water continuously for 1 min. The test group guinea-pigs were also sprayed continuously for 1 min. with a 1 : 80 aqueous solution of histamine acid phosphate (equivalent to 0.45% histamine base). The time (in minutes) at which each of the animals died (consequent to bronchospasm) was noted and recorded. Moreover, the number of survivors in each group was also recorded 20 min. following distilled water or histamine aerosol challenge. In another set of experiments, 10 adult control guinea-pigs and 10 other adult guinea-pigs per test compound were used. Each of the 10 guinea-pigs in the control group was pretreated with 0.5 ml distilled water, while the animals in the test groups were pretreated with either the plant extract (MESL, 50-800 mg/kg i.p.), aminophylline (50-800 mg/kg i.p.), or isoprenaline (50-800 μg/kg i.p.) 20 min. before they were exposed to distilled water and histamine aerosol challenges. In these *in vivo* experiments, aminophylline (50-800 mg/kg i.p.) and isoprenaline (50-800 μg/kg i.p.) were used as reference standards for comparison.

Data analysis

Results are expressed as means (±SEM). Where appropriate, the difference between "control" and "drug-treated" means was analysed statistically by using "Student's *t*-test". Values of $P \leq 0.05$ were taken to imply statistical significance.

Drugs used

The following drugs were used: acetylcholine chloride, histamine acid phosphate, potassium chloride (British Drug Houses); (−)-isoprenaline sulphate, aminophylline (Burroughs Wellcome); papaverine hydrochloride, (±)-propranolol hydrochloride (Imperial Chemical Industries); 5-hydroxytryptamine (Koch-Light); and methanolic extract of the root bark of *S. longipedunculata* (MESL). All drugs were dissolved or diluted in distilled water on each day of our experiment. Drug concentrations quoted in the *in vitro* experiments refer to the final organ-bath concentrations.

RESULTS

Guinea-pig isolated tracheal chain muscle preparations

Securidaca longipedunculata methanolic root bark extract (MESL, 50-800 mg/ml), like papaverine (50-800 μg/ml) or isoprenaline (0.1-1.0 μg/ml), produced concentration-dependent relaxations of the guinea-pig isolated tracheal chain muscle preparations contracted with acetylcholine (1 μg/ml), 5-hydroxytryptamine (0.6 μg/ml), histamine (1.5 μg/ml), or potassium (K^+, 30 mM). Fig. 1 shows a typical trace obtained with graded concentrations of MESL on a histamine-induced contraction of the guinea-pig isolated tracheal chain muscle preparation.

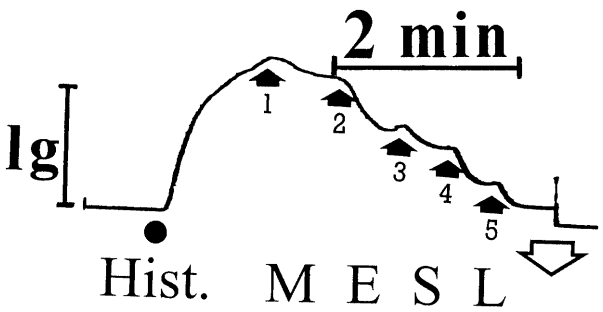

Fig. 1. Effects of graded concentrations of MESL on histamine-induced contractions of guinea-pig isolated tracheal chain muscle preparation. Histamine (1.5 μg/ml) was added to the bath fluid at the left-hand-side solid dot (Hist. ●). 1-5 right-hand-side solid upright arrows represent cumulatively administered MESL (50, 100, 200, 400 and 800 mg/ml respectively). Both histamine and MESL were washed out at the open right-hand-side downward pointing arrow.

The relaxant effects of MESL (50-800 mg/ml) and papaverine (50-800 μg/ml) on the muscle preparations were not affected by propranolol (0.5-1.0 μg/ml) which abolished or inhibited the relaxant effect of isoprenaline (0.1-1.0 μg/ml) on the muscle preparations. The rank order of potency of the spasmolytic agents examined was found to be: isoprenaline > papaverine > MESL. The tracheal muscle relaxing potencies of the compounds, calculated as concentrations of the agents that produced 50% of the maximal relaxations (i.e., IC_{50}), are presented in Table 1.

Histamine aerosol-induced bronchospasm in conscious guinea-pigs

None of the control (distilled water treated) guinea-pigs died 20 min. after exposure to distilled water spray. However, the normal unpretreated test guinea-pigs died 4-10 min. after exposure to histamine aerosol. Pretreatment of the test animals with either isoprenaline (50-800 μg/kg i.p.), aminophylline (50-800 mg/kg i.p.), or with the plant extract (MESL, 50-800 mg/kg i.p.), for 20 min. before histamine aerosol exposure markedly protected the guinea-pigs against histamine aerosol-induced bronchospasm. The order of potency of the test compounds in protecting the

Table 1. Comparison of the concentrations of papaverine, isoprenaline and MESL which produced 50% relaxation (IC_{50}) in the contractions of guinea-pig isolated tracheal chain muscle preparations induced by approximately equi-effective concentrations of acetylcholine (ACh), 5-hydroxytryptamine (5-HT), and histamine (Hist.)

Spasmogens (agonists)	Relaxant agents	Mean IC_{50} of Relaxant agents	Number of Observations
Histamine (1.5 μg/ml)	Papaverine	66±15 μg/ml	10
	Isoprenaline	0.47±0.05 μg/ml	10
	MESL	100±36 mg/ml	10
Acetylcholine (1.0 μg/ml)	Papaverine	61±12 μg/ml	9
	Isoprenaline	0.43±0.04 μg/ml	10
	MESL	120±35 mg/ml	10
5-Hydroxytryptamine (0.6 μg/ml)	Papaverine	70±18 μg/ml	10
	Isoprenaline	0.38±0.07 μg/ml	9
	MESL	112±40 mg/kg	10

Table 2. Comparison of the protective effects of graded doses of aminophylline, isoprenaline and MESL against histamine aerosol-induced bronchospasm *in vivo* in conscious guinea-pigs

Protective agents examined	Doses of protective agents administered i.p.	Number of guinea-pigs used	Number of survivors 20 minutes after histamine aerosol challenge	% Protection
Aminophylline	50 mg/kg	10	3	30
	100 mg/kg	10	5	50
	200 mg/kg	10	8	80
	400 mg/kg	10	10	100
	800 mg/kg	10	10	100
Isoprenaline	50 μg/kg	10	5	50
	100 μg/kg	10	7	70
	200 μg/kg	10	9	90
	400 μg/kg	10	10	100
	800 μg/kg	10	10	100
MESL	50 mg/kg	10	1	10
	100 mg/kg	10	3	30
	200 mg/kg	10	5	50
	400 mg/kg	10	7	70
	800 mg/kg	10	8	80

guinea-pigs against histamine aerosol-provoked bronchospasm was found to be: isoprenaline > aminophylline > MESL. The extent of protection offered by each of the three protective agents examined is calculated as "percentage protection" and presented in Table 2.

In some other experiments, adult guinea-pigs were pretreated with propranolol (200 μg/kg i.p.) 20 min. before parenteral administrations of either isoprenaline (50-800 μg/kg i.p.), aminophylline (50-800 mg/kg i.p.), or the plant extract (MESL, 50-800 mg/kg i.p.). Five propranolol-pretreated guinea-pigs were used for each dose of the test compounds. In all cases, the protective effect of isoprenaline on histamine aerosol-induced bronchospasm, but not that of aminophylline or MESL, was abolished or markedly inhibited by propranolol pretreament. This observation probably suggests the absence, or at best only very weak involvement, of β_2-adrenoceptor stimulation in the bronchospasmolytic effects of the plant extract (MESL) and aminophylline.

DISCUSSION AND CONCLUSION

The results obtained in this study show that *S. longipedunculata* root bark methanolic extract (MESL), like papaverine or isoprenaline, relaxed spasmogen-contracted guinea-pig isolated tracheal chain muscle preparations in a concentration-related manner. The plant extract also protected conscious guinea-pigs against histamine aerosol-induced bronchospasm. Although the exact mechanism of the bronchodilator action of the plant extract is still obscure, it is unlikely that the plant extract stimulates the β_2-adrenoceptors present on the bronchial muscles to produce bronchodilatation. This hypothesis is strengthened by the observations that (a) papaverine and aminophylline produced pharmacological effects that are similar to those of MESL in the experimental model used, and (b) propranolol which abolished or markedly inhibited the bronchospasmolytic effect of isoprenaline did not affect the bronchospasmolytic action of the plant extract.

Both sympathetic and parasympathetic components of the autonomic nervous system (ANS) play a role in the regulation of bronchial muscle tone. Sympathetic stimulation causes increased adenylate cyclase activity, and thus leads to a rise in the intracellular concentration of cyclic adenosine monophosphate (cAMP). On the other hand, parasympathetic stimulation causes, via increased guanylate cyclase activity, a rise in the intracellular concentration of cyclic guanosine monophosphate (cGMP). Stimulation of pump mechanisms produces either a reduction in intracellular calcium, and thus tracheal muscle relaxation, or an increase in intracellular calcium, and thus tracheal muscle contraction. An increase in the intracellular concentration of cAMP inhibits the release of chemical mediators of bronchial asthma, and thus facilitates bronchodilatation. In contrast, an increase in the intracellular concentration of cGMP stimulates chemical mediator secretion and/or release, and consequently promotes bronchoconstriction. In any type of asthma, the patient is reacting to an internal or external stimulus which is usually harmless to a normal subject. This belief has led to the concept of bronchial hyperreactivity as the basic abnormality present in all asthmatics. The therapeutic implication of this concept is that prophylactic treatment of bronchial asthma should be aimed at reducing bronchial hyperreactivity.

The vagus is the dominant nerve supply to the bronchial smooth muscle. Stimulation of this parasympathetic nerve leads to the release of acetylcholine (ACh) at the post-ganglionic nerve terminal. The acetylcholine thus released from the post-ganglionic nerve terminal will combine with, and stimulate post-junctional M_3-muscarinic cholinoceptors on the bronchial smooth muscle, resulting in bronchoconstriction. Acetylcholine release from pre-junctional nerve terminals is regulated by autoinhibitory M_2-muscarinic cholinoceptors. Blockade of these pre-junctional M_2-cholinoceptors leads to facilitation of nerve-mediated bronchoconstriction, whereas blockade of the ganglionic nicotinic cholinoceptors, or the post-junctional M_3-cholinoceptors, will reduce nerve-mediated bronchoconstriction. Recent studies in our laboratories (Ojewole et al., 2001) have shown that methanolic extract of *Securidaca longipedunculata* root bark possesses post-junctional anticholinergic, antihistaminergic, and antiserotonergic properties. In all preparations examined, the plant extract also inhibited the spasmogenic and/or stimulant actions of potassium (K^+) and calcium (Ca^{2+}) in a non-specific manner. It is likely, therefore, that the antibronchoconstrictor effect of the plant extract is mediated largely through its ability to antagonise the post-junctional spasmogenic actions of acetylcholine, histamine, and other bronchial smooth muscle spasmogens and stimulants.

It has been reported that the root bark of *S. longipedunculata* contains volatile oils with large amounts of methylsalicylate, as well as various sapogenins (including presenegenin), toxic indole alkaloid securinine, and some ergot alkaloids [Van Wyk et al., (1997)]. Since saponins and sapogenins are known to possess non-specific spasmolytic properties against a wide range of spasmogens, it is not unlikely, therefore, that the bronchodilator activity of the plant may be attributed largely to presenegenin, a triterpenoid sapogenin present in the plant extract.

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