Medicinal plants in Suriname: hypotensive effect of *Gossypium barbadense*

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Abstract

In traditional medicine *Gossypium barbadense* L. is used against hypertension. Looking for a scientific basis for this use, the blood-pressure-lowering effect of the decoction of the leaves was confirmed. Fraction II (frII) of the crude extract of *G. barbadense* showed a dose-dependent hypotensive effect in anaesthetized rats. In hexamethonium-treated rats, the blood-pressure-lowering effect of frII was almost abolished. A small decrease of the blood-pressure-lowering effect was followed by an increase in the blood pressure. Phentolamine antagonized the increase in blood pressure in hexamethonium-treated rats. High doses of atropine (4 mg/rat) suppressed both depressor and heart effects. In-vitro experiments revealed that atropine did not antagonize the contraction of the ileum of the rat. Tripelennamine in a concentration of 100 μg could not influence the contraction either, whereas 300 μg did. In the guinea-pig ileum 10 μg tripelennamine did not reduce the contraction significantly.

In the mechanism of action of frII, acetylcholine receptors could be involved, but not histaminergic or adrenergic receptors. Although it is still not known which compound(s) in *G. barbadense* is (are) the active substance(s), the results obtained may explain the use of this plant in traditional medicine in Suriname.

Introduction

The application of medicinal plants and herbal remedies has become popular worldwide and is increasing both in developing and developed countries. About 20 000–40 000 plants are used for medicinal purposes; most of these are used in traditional systems of medicine as well as in the cosmetics, nutrition and essential oils industry (Hasrat 1997). Nowadays, scientific research into medicinal plants is carried out on a larger scale than ever before. The main reason is that, notwithstanding the great progress in biotechnology, there has not been a breakthrough to produce better, specific and safe drugs.

In Suriname, located on the northern coast of South America and rich in natural resources, the application of traditional medicine has been known since the discovery of the Guyanas. All of the ethnic groups from other continents that have settled in Suriname have added to the rich and various traditional medicine culture that exists now. There is, however, a lack of scientifically based information on traditional medicine in this country, especially regarding medicinal plants. The scientific investigation of medicinal plants in Suriname was based on information gathered in the publication of Heyde (Hasrat 1997).

Many inhabitants in the interior of Suriname, and even urban inhabitants, are deprived of a good and regular public health service, due to lack of proper communication and transport and the economical situation of the country. Reliable information about the natural products of Suriname and traditional medicine can be used by public health workers and in general by all inhabitants, for the treatment of common diseases. Therefore, in light of the recognized value of medicinal plants in the treatment of several diseases, much more scientific investigation must be conducted (Hoareau & DaSilva 1999; Ernst 2003).

In Suriname, although it is a developing country, the prevalence of hypertension is relatively high. The impression is that the incidence is the highest among those descended from African origin, although this has not been confirmed scientifically. It is by these

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people that herbal products are frequently used, as part of their traditional medicine, for the treatment of symptoms that might be due to hypertension. *Gossypium barbadense* L. (vernacular name, redi katoeng or red cotton), a member of the Malvaceae family, is one of the plants used for this purpose (Morton 1981; Heyde 1985). A decoction or an infusion of the leaves from this plant is used.

This investigation has been initiated with the objective of finding a scientific basis for the claim that *G. barbadense* lowers blood pressure. Elucidation of the pharmacological mechanism might provide new insight into the pathophysiology and the treatment of hypertension. Isolation of active components could then lead to the development of new therapeutics. Due to the worldwide renewed interest in medicines of natural origin, the development of a suitable therapeutic from *G. barbadense* may have economic benefit for Suriname.

The blood-pressure-lowering effect of *G. barbadense* has never been described before in scientific literature. There has not been a thorough description of the phytochemistry of the plant. Gossypol, a toxic (carcinogenic) compound, as well as some other compounds, has been detected in cotton seed (Morton 1981).

In the first report we have focused on the ethno-pharmacological claim and in the near future we hope to present the isolation of the active compound(s).

Materials and Methods

Materials

Materials used were: frII (500, 1500, 5000 μg mL^{-1}); isoprenaline (10 μg mL^{-1}); noradrenaline (epinephrine) (10 μg mL^{-1}); hexamethonium (25 mg mL^{-1}); acetylcholine (1500 μg mL^{-1}); phentolamine (10 mg mL^{-1}); tripelennamine; BaCl$_2$ (2 mg mL^{-1}); urethane; heparin; histamine; and solutes for different buffers. Every test solution was prepared in buffered salt solution, pH 7.4.

Plant extracts

Gossypium barbadense L. (Malvaceae), identified by M. Werkhove from the herbarium of the University of Suriname, where a voucher specimen is kept, was collected in one of the dry seasons (October/November) in a village about 20 kilometers from the Surinamese capital, Paramaribo.

The nervation of the leaves was removed, the leaves were then washed in distilled water, crushed in a blender and centrifuged at 8000 rev min^{-1}. The liquid was evaporated under reduced pressure at a temperature of 50 °C, almost to dryness. Finally, the concentrated solution was frozen in liquid nitrogen and freeze-dried.

Purification procedure

Column chromatography with Al$_2$O$_3$ neutral (super I activity), obtained from Merck, was the first step in the purification of the decoction, after it was established that the blood-pressure-lowering effect was still present when column chromatography was performed with neutral and acid Al$_2$O$_3$, and not with basic Al$_2$O$_3$. The elution was performed with water. The polar compounds present in this eluate were subjected, after concentration, to high-pressure liquid chromatography (HPLC) on a semi-preparative reversed-phase column (10 μm). The elution was performed with 20% methanol and subsequently pure methanol, using a flow speed of 4 mL min^{-1} with UV (254 nm) detection. The 20% methanol eluate was separated after 1 min 42 s in two fractions (fraction I (frI) and fraction II (frII)), and methanol eluate was collected as one fraction (frIII). After evaporation of methanol in the three fractions, under reduced pressure, the remaining water was freeze-dried. Quantities of the three fractions were dissolved in buffered salt solution, pH 7.4, and the test solutions were obtained through dilutions with the same buffer.

Experimental protocols

The in-vivo pharmacological experiments were performed to measure the blood-pressure-lowering activity of the crude extract and purified fractions of *G. barbadense* and to investigate the site of action (hexamethonium experiments) and the mechanism of action (atropine experiments), whereas the in-vitro experiments (histamine experiments) were performed to investigate only the mechanism of action. The blood-pressure-lowering activity was used as method for the bio-guided isolation of active compounds.

The animals used in the experiments were kept under controlled lighting conditions in a temperature-controlled environment (22 °C). The animal quarters were illuminated from 0600 to 1800 h. Food and water were freely available.

In-vivo experiments

Wistar rats, 180–280 g, were used. The blood-pressure experiments were performed using anaesthetized rats. Urethane was used as the anaesthetic; 0.1–0.2 mL/100 g rat from a 160 g/100 mL urethane solution was injected intraperitoneally. Catheters containing heparinized (50 U mL^{-1}) saline were inserted into the right carotid artery and the right external jugular vein for blood-pressure measurement and intravenous administration of drugs, respectively. The arterial catheter was connected to a Statham P23AA pressure transducer and mean arterial blood pressure was derived from the direct measurement. The pressure transducer was connected to a Sandborn pre-amplifier and the blood pressure was subsequently registered on a Sandborn recorder. The recorder was calibrated before every experiment in which the blood pressure was measured in mmHg. The heart frequency was derived from the blood pressure recording or by electrodes connected to the paws of the rat.

In each experiment control measurements were initially performed. First, the application of buffered salt solution, pH 7.4, followed by 10 μg of isoprenaline, a non-selective β-adrenergic receptor agonist, and noradrenaline (norepinephrine), respectively, to verify if the cardiovascular

system was responding to blood-pressure-active substances. Next, the different dosages of frII were applied at random.

In the hexamethonium experiments, artificial respiration was used before hexamethonium (20 mg), a blocker of ganglia of the autonomic nervous system, was applied. Thereafter, several dosages of frII were applied at random.

In the atropine experiments, the procedure was followed by the application of acetylcholine (150 μg). Atropine (4 mg), an antagonist of the muscarinic-type receptors of acetylcholine, was then applied followed by the same dosage of acetylcholine to check the blockade of the muscarinic receptors. Thereafter, the different dosages of frII were applied at random.

At the end of every experiment, 10 μg of isoprenaline and noradrenaline, respectively, were again applied to investigate changes in the cardiovascular performance of the rat. The application of any substance in the experiments was made after the blood pressure had returned to the value before the application or had stayed constant at that or another value for at least 5 min.

In-vitro experiments

Ileum of rats, 200–280 g, and one guinea-pig, 300 g, were used. The animals were killed by a blow on the head and cutting the throat. Through a midline section of the abdomen, a piece of terminal ileum was cut off and rinsed with Tyrode's buffer (Perry 1970). This piece of ileum was further divided into smaller pieces with which the experiments were performed. The perfusion fluid was oxygenated Tyrode's buffer that was applied from the reservoir and warmed to 37 °C by water circulating from a thermostat. For isotonic contraction measurements, one end of a piece of ileum was attached by a thread to a fixed pin in the glass organ-bath, the other end was attached by a thread to an iron rod, which was further connected by a thread, walking over a wheel, to a fixed load (1 g). The iron rod moved in a magnetic field when the length of the piece of ileum changed. These movements caused small electrical currents that were amplified by a Sandborn pre-amplifier and registered on a Sandborn recorder. The change of the length of the organ was expressed as the change in mm on the recorder at a fixed attenuation level.

Each test material was administered in volumes of 0.1 mL to the organ-bath solution. Before the application of atropine the ileum was washed three times, and after each administration of atropine the ileum was washed five times.

After application of buffered salt solution, the different dosages of frII used in these experiments were applied at random. Acetylcholine, 100 μg, was then applied followed by 10 μg of atropine and 100 μg of acetylcholine, the latter to check muscarinic receptor blockade. Next, 10 μg of atropine was applied to the organ bath solution before each of the dosages of frII was administered.

Results

After confirming the blood-pressure-lowering effect of the crude extract of *G. barbadense* and the neutral Al_2O_3

column elution sample, HPLC reversed-phase column chromatography fractionation was performed. Three fractions were collected – two 20% methanol fractions and one methanol fraction.

Blood-pressure-lowering effect of frII

There was a dose-dependent decrease of the blood pressure with frII in anaesthetized rats (Table 1).

Hexamethonium experiments

These experiments were conducted to investigate the site of action (central nervous system (CNS) or peripheral) of frII. The results (Table 2; Figure 1) showed that hexamethonium abolished the effect of frII (Table 2, column III); moreover, frII had opposite effects (Table 2, column IV). The increase of blood pressure in the presence of hexamethonium was dose dependent and was suppressed by phentolamine, a non-selective α-adrenergic receptor antagonist (results not presented). Phentolamine was introduced after the last application of frII in the hexamethonium experiments.

Atropine experiments

Atropine (10 μg) completely antagonized the blood-pressure-lowering effect of acetylcholine, decreased the effect of isoprenaline significantly and diminished the effects of the doses of frII (Table 3). Moreover, in the presence of atropine, frII caused first a small decrease in blood pressure followed by an increase. The frII dose-dependent decrease of the heart frequency was also completely antagonized by atropine.

Interaction of propranolol and phenoxybenzamine in rats with the action of frII

In a few experiments conducted with frII (results not shown) in the presence of propranolol, a non-selective β-adrenergic receptor antagonist, it was observed that

Table 1 Dose–response relation of frII in anaesthetized rats.

Compound	Δ Blood pressure (mmHg)
1 μg Isoprenaline	−42 ± 10
1 μg Noradrenaline	47 ± 9
Control	−2.5 ± 1.1
5 μg frII	−1 ± 1
15 μg frII	−5 ± 6
50 μg frII	−15 ± 5*
150 μg frII	−26.3 ± 6.0
500 μg frII	−36 ± 13**

Anaesthesized rats, initial blood pressure 124 ± 11 mmHg, received frII dosages at random after the response to administration of isoprenaline and noradrenaline was determined. Data are means ± s.e.m., n = 6. Analysis of variance one-pair test was applied; *$P < 0.05$, versus control and 50 μg frII; **$P < 0.05$, versus 50 μg frII and 500 μg frII.

Table 2   Hexamethonium experiments in anaesthetized rats: determination of the site of action of frII.

	Before hexamethonium application		After hexamethonium application			
	Δ Blood pressure (mmHg)	Δ Heart frequency (beats per min)	Δ Blood pressure (mmHg)	Δ Heart frequency (beats per min)		
	I	II	III	IV	V	VI
1 μg Isoprenaline	−54.2 ± 4.4	+24 ± 10	−23.3 ± 3.5			
1 μg Noradrenaline	+29.7 ± 1.3	−4 ± 3				+55.6 ± 2.6
Control	0	0	0	0	0	0
50 μg frII	−36.0 ± 2.3	−4 ± 2	−5 ± 3.6	0.8 ± 0.8	−1 ± 1	+1 ± 1
150 μg frII	−45.3 ± 2.5	−14 ± 3	−3.9 ± 3.4	+17.7 ± 2.2	−3 ± 2	+6 ± 3
500 μg frII	−47.7 ± 2.2	−70 ± 23	−4.0 ± 4.0	+38.3 ± 3.3	−2 ± 2	+16 ± 8

Anaesthetized rats, receiving artificial respiration after treatment with hexamethonium (20 mg), were administered frII dosages at random. Data are means ± s.e. m., n = 6. The data were compared with those before hexamethonium treatment. The initial blood pressure and heart frequency before introduction of hexamethonium were 127.3 ± 3.2 mmHg and 372 ± 40 beats per min, respectively. After treatment with hexamethonium these were changed significantly ($P < 0.05$) to 79.2 ± 7.8 mmHg and 302 ± 14 beats per min, respectively. Analysis of variance one-pair test exhibited a significant difference ($P < 0.05$) between the responses to frII dosages before hexamethonium, except 150 μg frII vs 500 μg frII. After treatment with hexamethonium, the frII responses were significantly different ($P < 0.01$), as before treatment. No significant differences were measured ($P > 0.05$) between the blood-pressure-lowering effects of the frII dosages, but the blood-pressure-increasing effects of the frII dosages were significantly different ($P < 0.05$) after treatment with hexamethonium.
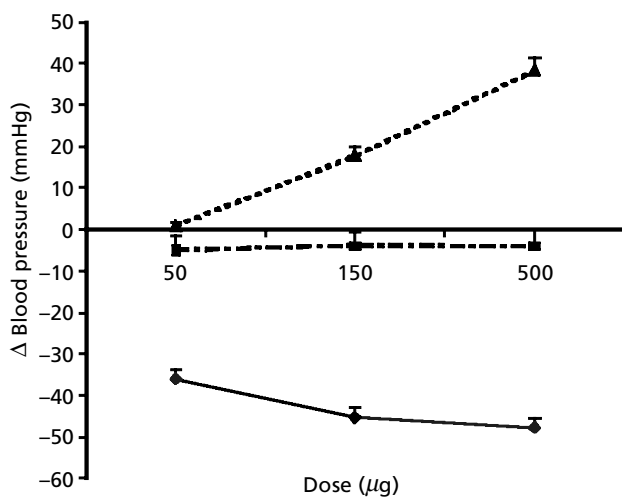

Figure 1   Site of action of frII: influence of hexamethonium on the effects of frII in anaesthetized rats. ◆, frII; ■, frII decrease in blood pressure after hexamethonium; ▲, frII increase in blood pressure after hexamethonium. The blood-pressure-decreasing effects of frII in the presence of hexamethonium have changed significantly into first a non-dose-dependent much lesser decrease in the blood pressure and thereafter a dose-dependent blood-pressure-increasing effect that is shown separately. The data are presented as means ± s.e.m., n = 6.

the effects of frII (50 μg and 150 μg) were not reduced after addition of both 100 μg and 200 μg propranolol. Phenoxybenzamine, a non-competitive, non-selective α-adrenergic receptor antagonist, 100 μg/100 g intraperitoneally, administered 20 h and 1 h before administration of frII (50 μg and 150 μg), did not influence the effect of frII, but completely abolished the blood-pressure-increasing effect of adrenaline.

In-vitro experiments

Isolated ileum experiments
Atropine did not antagonize the effects of frII on the ileum of the rat (Table 4). Instead, it potentiated the contraction of frII. Although tripelennamine antagonized the effects of frII on rat ileum (Table 4), the number of experiments was too small to draw any conclusion. In guinea-pig ileum, the dose–response relation of frII showed a biphasic pattern, different from that of histamine. Moreover, the guinea-pig ileum was much less sensitive to frII than histamine (Figure 2).

Discussion

The use of phytotherapeutics is increasing worldwide. So, the need for scientifically collected data on these products is in conjunction with these demands. Ignoring these facts is wrong and does not help to collect reliable information on these therapeutics for the public health in general (Ernst 2003). This study was, therefore, conducted to find scientific evidence for the claims of traditional medicine practitioners that red cotton, *Gossypium barbadense* L., possesses blood-pressure-lowering effects. In addition, the isolation of active compounds could lead to new effective therapeutics for the treatment of hypertension.

From the crude extract of *G. barbadense*, which previously had shown blood-pressure-lowering effects in anaesthetized rats, frII was collected as the most active

Table 3 Mechanism of action: interaction of atropine with the cardiovascular effects of frII in anaesthetized rats.

	Δ Blood pressure (mmHg)		Δ Heart frequency (beats per min)	
	Before atropine	After atropine	Before atropine	After atropine
1 μg Isoprenaline	−48.2 ± 4.2	−12.2 ± 4.1	+13 ± 5	+6 ± 4
1 μg Noradrenaline	+29.2 ± 4.4		−8 ± 5	
Control	−0.7 ± 0.7	−1 ± 0.8	0	0
50 μg frII	−27.2 ± 5.7	−6.8 ± 4.5	−2 ± 2	−2 ± 2
		+0.3 ± 0.3		
150 μg frII	−35.7 ± 5.1	−9.5 ± 6.0	−26 ± 19	−2 ± 2
		+2.2 ± 1.6		
500 μg frII	−43.2 ± 3.7	−5.4 ± 3.3	−106 ± 48	+7 ± 7
		+8.6 ± 2.8		
150 μg Acetylcholine	−64.2 ± 2.8	−2.3 ± 2.3	−273 ± 18	0
		+4.0 ± 2.8		

After anaesthetized rats had received atropine (4 mg), the blood-pressure-lowering effects of frII were changed significantly ($P < 0.01$; analysis of variance one-pair test). Data are means ± s.e.m., n = 6. The initial blood pressure decreased significantly ($P < 0.01$) from 110.8 ± 4.9 to 73.2 ± 9.1 mmHg. The heart rate was not influenced, 331 ± 17 beats per min before, and 328 ± 17 beats per min after atropine.

Table 4 Mechanism of action of frII: effects of frII in the ileum of the rat after treatment with atropine.

	Change in ileum length (mm)	
	Before atropine	After atropine
150 μg frII	16 ± 5	25 ± 5
100 μg Acetylcholine	90 ± 10	0

Rat ileum preparations (see text for more details) were exposed to frII before and after treatment of atropine (10 μg). The response to frII was increased, but not significantly ($P > 0.05$). The effect of acetylcholine was completely inhibited. The change in the length of the ileum was expressed as the change in mm on the recorder paper at a fixed attenuation level of the amplifier.

Figure 2 Mechanism of action: dose–response curves of histamine and frII on the guinea-pig ileum in the presence and absence of tripelennamine. ◇, histamine; □, histamine after administration of tripelennamine; △, frII; ×, frII after administration of tripelennamine. Guinea-pig ilea were exposed to increasing dosages of frII and histamine with or without tripelennamine (10 μg), a histaminergic receptor antagonist. The response to histamine was significantly changed ($P < 0.05$; Student's t-test), in the presence of tripelennamine and the curve was shifted to the right. The response of the tissue to frII was much smaller than to histamine and was not significantly changed in the presence of tripelennamine. The change in the length of the ileum was expressed as the change in mm on the recorder paper at a fixed attenuation level of the amplifier. The data are presented as means ± s.e.m., n = 6.

fraction after HPLC purification procedure. The pharmacological data presented here were collected from experiments with this HPLC fraction.

This study was carried out following a classical pharmacological approach. First, the interaction between active compound(s) in frII and a receptor was established through investigation of the dose dependency of the response. The response to a ligand is proportional to the number of receptors occupied by the ligand at equilibrium (Williams 1991). The dose–response relation of frII (Table 1) gives hard evidence that in the extract of *G. barbadense*, at least one compound with a blood-pressure-lowering effect is present. Second, the site of action was investigated through the experiments with hexamethonium, which showed that, with regard to the blood-pressure-lowering effect of frII, the receptor is likely to be present in the CNS. The physiological effects after administration of hexamethonium can
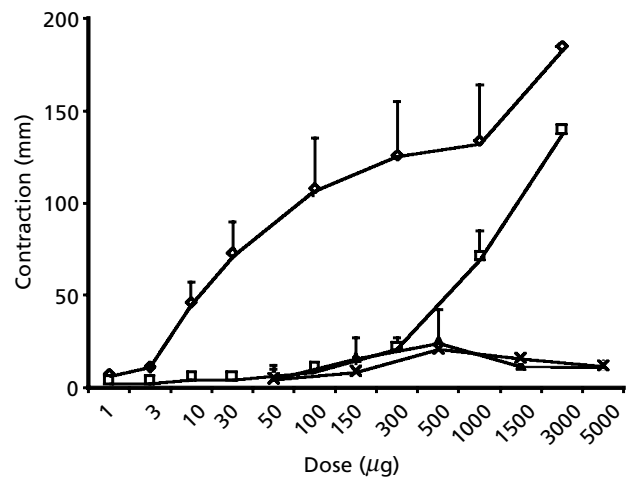
be attributed to the blockade of transmission in ganglia of the autonomic system (Taylor 1980; Rang et al 1999a). Centrally acting antihypertensive drugs produce their effects

by reducing activity of the sympathetic nervous system (Rang et al 1999b; Blaschke & Melmon 1980). The bradycardia produced after application of frII (Tables 2 and 4) indicated that the active compound acts in the CNS. There is also a blood-pressure-increasing effect that is evident when the blood-pressure-lowering effect is masked. The blood-pressure-increasing effect is dose dependent, as is depicted in the hexamethonium experiments (Figure 1). This action is antagonized by phentolamine, an α-adrenergic antagonist. The effects of frII on the blood pressure resemble the action of clonidine; however, it is not excluded that the effects of frII might be produced by more than one compound. Clonidine has a blood-pressure-lowering effect, through action in the CNS, and a blood-pressure-increasing effect, via α-adrenergic receptors in the vessels (Blaschke & Melmon 1980).

The investigation of the mechanism of action of active compound(s), as the third part of the classical pharmacological approach, revealed that β-adrenergic and α-adrenergic receptors are not involved. β-adrenergic receptor stimulation in the vessels produces vasodilatation and, consequently, a decrease in the blood pressure. Stimulation of α_1-adrenergic receptor increases the blood pressure, while stimulation of α_2-adrenergic receptors decreases the blood pressure. In a number of experiments, performed with propranolol and phenoxybenzamine, it was shown that these compounds did not exhibit any antagonistic action on the blood-pressure-lowering effect of frII. In those experiments, the blood-pressure-lowering effect of isoprenaline and the blood-pressure-increasing action of adrenaline were completely antagonized by propranolol and phenoxybenzamine, respectively, which implies that none of the peripheral β-adrenergic and α-adrenergic receptor types are involved in the blood-pressure-lowering effect of frII. The α-adrenergic receptor types seem to be involved in the blood-pressure-increasing effect of frII, because phentolamine, a competitive α-adrenergic receptor antagonist, depressed this effect.

Further, atropine diminished the blood-pressure-lowering effects of frII, although the effect of isoprenaline was also depressed. This antagonistic activity may be due to action of atropine on the CNS, where a circuit that controls blood pressure is influenced.

In-vitro experiments have shown dual effects of frII; frII produces contraction in rat and guinea-pig ileum; however, in the latter preparation a steady decrease of the contraction at the highest doses is observed (Figure 2).

Atropine did not decrease the contraction produced by frII at the ileum of the rat. The interaction with histaminic receptors found in the experiments with tripelennamine revealed an antagonistic activity with high doses of tripelennamine, although the effects of frII on the ileum of the rats were as small as with histamine.

The results of the experiments with guinea-pig ileum showed a biphasic effect of frII. Tripelennamine interfered with, but did not reduce completely, the contraction produced by frII. The combination of tripelennamine and atropine gave an increased reduction of the effects of frII on this preparation.

In the light of the above results, it may be concluded that the extract of *G. barbadense* has blood-pressure-lowering

effects and so there must be at least one active compound present in the extract. The blood-pressure-lowering effect is produced through an action on the CNS. Moreover, the effect on blood pressure is comparable with that of clonidine, an α_2-adrenergic receptor agonist (Blaschke & Melmon 1980). The mechanism of action of the active compound(s) must still be elucidated. Interaction with muscarinic receptors seemed to play a role, as was demonstrated with the atropine experiments; however, an interaction with histaminic receptors is not excluded. Further, an interaction with adrenergic receptors could not be demonstrated.

More research has to be carried out to elucidate the mechanism of action of the extract. Radioligand binding studies, as performed with other plants (Hasrat et al 1997a, b, c, d, e), may contribute to solve this, whereas the isolation of the active compound(s) will make the interpretation of the results easier.

This is the first publication in which the blood-pressure-lowering effect of *G. barbadense* has been described, as is claimed in the traditional medicine.

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