BLOOD PRESSURE LOWERING EFFECT OF THE ETHANOL EXTRACT FROM THE STEMBARK OF *CINNAMOMUM ZEYLANICUM* (LAURACEAE) IN RATS

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Summary

Cinnamomum zeylanicum (Lauraceae) bark is generally used in Cameroon ethnomedicine for the treatment of cardiovascular diseases. Intravenous (i.v) injection of the ethanol extract resulted in a biphasic dose-related hypotensive activity. In normotensive rats (NTR), *C. zeylanicum* at the doses of 5, 10 and 20 mg/kg decreased systolic blood pressure (SBP) an hour after administration by 10.51 %, 17.55 % and 19.06 %, respectively. In salt-loaded hypertensive rats (SLHR), the decrease in systolic blood pressure was 13.10 %, 20.87 %, and 30.54 % at the above doses, respectively. This late hypotensive effects might be due to the reduction of peripheral resistance. In order to verify this hypothesis, the extract was assayed for its vasorelaxant activity. The results showed a vasorelaxant effect of *C. zeylanicum* on the rat thoracic aortic ring segments with (+E) or without (-E) endothelium precontracted with KCl (60 mM), suggesting that, *C. zeylanicum* might be inhibiting extracellular Ca^{2+} through L-type voltage-sensitive channels. These findings suggest that *C. zeylanicum* exhibited it first hypotensive effect probably by reducing cardiac activity and secondly through vasodilation.

Key words: *Cinnamomum zeylanicum*, hypotensive effect, rat

Introduction

Cinnamomum zeylanicum (Lauraceae) is a small tree native of Sri Lanka (1). The barks are commonly used as spices in some parts of Cameroon. They are also used in traditional medicine to treat male impotence, digestive disorders, and hypertension, but also to ease delivery in childbirth. *C. zeylanicum* is a small tree growing up to 2 m high with an upright stem. The barks are brown reddish; the leaves are bright green, marked with three main parallel veins, the flowers are white and always grouped into three (2). Phytochemical studies have revealed the presence of phenolic constituents in the fruits (3) and some chemical constituents such as (E)-Cinnamyl acetate, trans-alpha-bergamoten and caryophyllen oxide in the flower oil (4).

The aim of this study was to evaluate by the invasive method the hypotensive properties of the ethanol extract, using two experimental rats' models namely, normotensive rats and salt-loaded hypertensive rats and to determine the possible mechanisms involved in the blood pressure lowering effect.

Materials and Methods

Animals

The experiments were carried out on normotensive and salt-loaded hypertensive adult rats, aged 12 to 15 weeks and weighing 170 to 250 g. The rats were raised in the animal house of the faculty of science, University of Dschang, Cameroon and fed on normal laboratory rat died (Granules) with water given ad libitum. Salt-loaded hypertensive rats were obtained from normotensive Wistar rats. Young normotensive Wistar rats weighing 80-120 g (4-5 weeks) were given by gastric intubation once a day, a solution containing 9 % NaCl at 2 ml/100 g body weight with free access to normal rat chow and 1,2 % NaCl solution as drinking water. After 8-9 weeks of treatment, all treated rats were tested and confirmed as hypertensive with blood pressure averaging 140 to 160 mmHg.

Preparation of the plant extract

The plant material was harvested in Nkondjock, Cameroon between March and April 2005. The harvested fresh barks were sun dried and ground into a fine powder. 300 g of the powder were macerated in ethanol (95%) for 48 hours. The filtrate was concentrated under reduced pressure at 70°C to give 42 g, corresponding to an extraction yield of 12 %. 300 mg of the extract were dissolved in 0.5 ml of dimethyl sulphoxide (DMSO) and the volume of solution was made up to 20 ml using distilled water. Dilution was later made according to

the experiment and dependent of the dose. The effects of solvent (2.5 % DMSO) were tested in order to ascertain that the results obtained were exclusively due to the extract (5).

Phytochemical screening

The use of the test for the presence of flavonoids, alkaloids and polyphenols was done using the technique described by Richard, (1998), while the presence of saponins were revealed as described by Hosttetmann, et al (1997).

Animal Preparation for Blood Pressure measurement

The rats were anaesthetized by the intraperitoneal injection of 0.1 ml/100 g body weight of Thiopental at a dose of 50 mg/kg. The femoral vein was canulated for the administration of plant extract and reference drugs while the carotid artery was also canulated for the blood pressure measurement using a blood pressure transducer model Ugo Basile PRC 21k-10. The preparations were allowed to stabilise for at least 30 min before administration of a test substance. Blood pressure variation was detected and recorded on an Ugo Basile Unirecord model 7050.

Aorta Preparation

Aortic rings were obtained from normotensive Wistar strain rats (170-250 g) after cervical dislocation. The thoracic aorta of the rat was carefully excised and, devoid of fat and connective tissues and later place in dissecting dish containing physiological salt solution (PSS) of the following composition (in M). NaCl, 6.96 g; KCl, 0.35 g; $CaCl_2$, 0.28g; $MgCl_2$, 0.11 g; $NaHCO_3$, 1.18 g, KH_2PO_4, 0.16 g and glucose, 2 g; 1l. A segment of the aorta was cut helically into longitudinal strips of about 10 mm length. In some experiments, the aorta was denuded of endothelium by gently rubbing the luminal surface with cotton. The strips were then suspended in a tissue bath of 20 ml of PSS maintained at 36°± 0.5°c, pH 7.4 and bubbled with air. CO_2 was removed from the air by passing it through a saturated NaOH solution. The mechanical activity was recorded isometrically using a force displacement transducer (Ugo Basile) connected to a recorder. The initial resting force applied to the aorta strip was 1.0 g. Each preparation was allowed to equilibrate for at least 60 min prior to initiation of experimental tests. During this period, the incubation media were changed every 15 minutes.

Drugs used

Atropine sulphate from Guangdong Medicines & Health Products, Belgium. Heparin from Sanofi, France. Propranolol from Sigma chemical, ST Louis, MO, USA. All drugs were dissolved in distilled water except for the plant extract that was dissolved in dimethyl sulphoxide (DMSO) and the solution adjusted with distilled water.

Statistical analysis

Data are presented as mean ± standard error of the mean. The percentage changes in SBP of NTR or SLHR over the 60 min were evaluated with ANOVA repeated measurement followed by Newman-Keuls Multiple comparison test using Graph Pad prism 3.0. In the organ bath studies, the EC_{50} value was calculated using Graph Pad prism.

Results

Phytochemical screening

The phytochemical screening revealed in the ethanol extract the presence of flavonoids, alkaloids and polyphenols. The presence of saponins was also found in the extract.

Effects of the ethanol extract of *C. zeylanicum* on blood pressure

Effects observed in normotensive rats (NTR)

The intravenous administration of the ethanol extract of *C. zeylanicum* provoked in anaesthetized NTR an immediate fall of blood pressure. SBP dropped from 129.06 ± 6.65 mmHg to 112.26 ± 8.20 mmHg at a dose of 5 mg/kg, from 133.60 ± 9.23 mmHg to 121.20 ± 8.20 mmHg at a dose of 10 mg/kg and from 123.20 ± 4.63 mmHg to 110.80 ± 5.85 at a highest dose of 20 mg/kg, representing a drop of 11.15%, 8.79% and 10.26% respectively. Figure1 shows the time course of the hypotensive action following i.v injection at the doses of 5-20 mg/kg. As shown, the immediate fall in blood pressure induced by the extract was followed by a sustained and significant hypotensive effect that lasted for more than one hour.

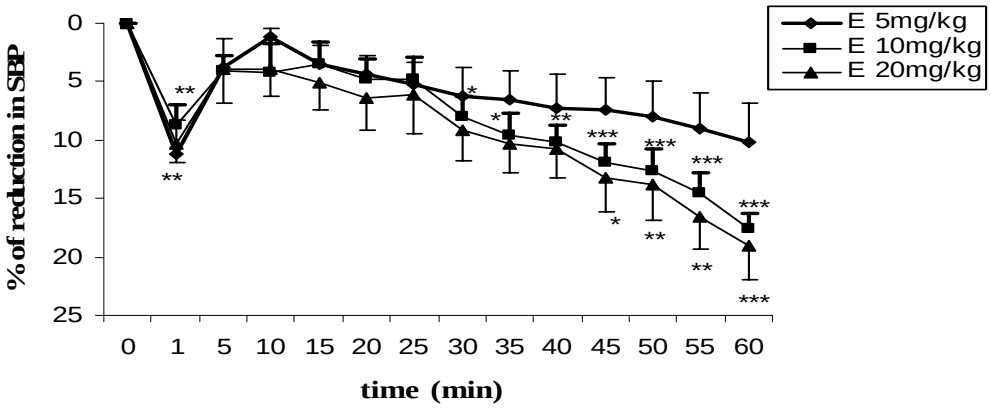

Figure 1: Effect of the ethanol extract of *C. zeylanicum* (E) on the systolic blood pressure in normotensive rats. Each point represents the mean ± SEM (n=5)
*p<0.5, **p<0.01 and ***p<0.001 significantly different compared to the initial value.

Effects on salt loaded hypertensive rats (SLHR)

In SLHR, the ethanol extract produced a dose-dependent fall in blood pressure. It induced a more significant biphasic hypotensive effect than in NTR. There was no significant difference between the first responses induced on SBP by the ethanol extract. Although the second phase started 15 min after administration, the latter falls in SBP was more pronounced and were 13.10%, 20.87% and 30.54% respectively at doses 5, 10 and 20 mg/kg (fig 2).

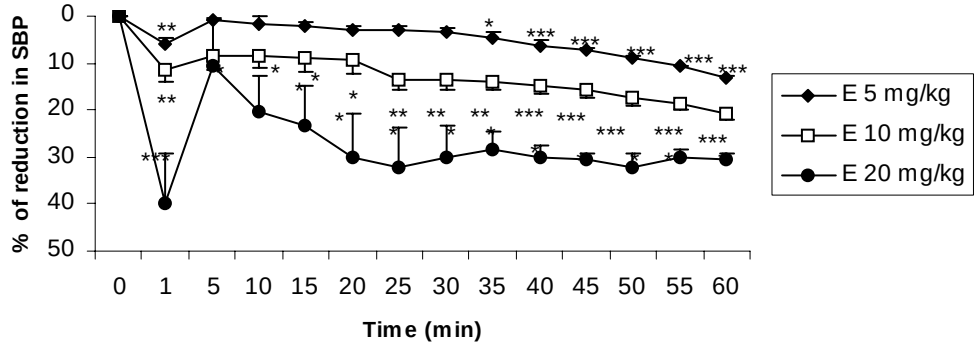

Figure 2: Effect of the ethanol extract of *C. zeylanicum* (E) on the systolic blood pressure in salt-Loaded hypertensive rats. Each point represents the mean ± SEM (n=5)
*p< 0.05, **p< 0.01 and ***p<0.001 significantly different compared to the initial value.

Further studies were carried out to elucidate the possible mechanisms by which *C. zeylanicum* bark extract elicited the observed hypotensive effect. The plant extract was given 5 min after the administration of propranolol (1mg/kg) or atropine (1mg/kg). It was found that propranolol had potentiating effect on the initial response provoked by the extract while atropine potentiated the initial and late hypotensive response (fig 3)

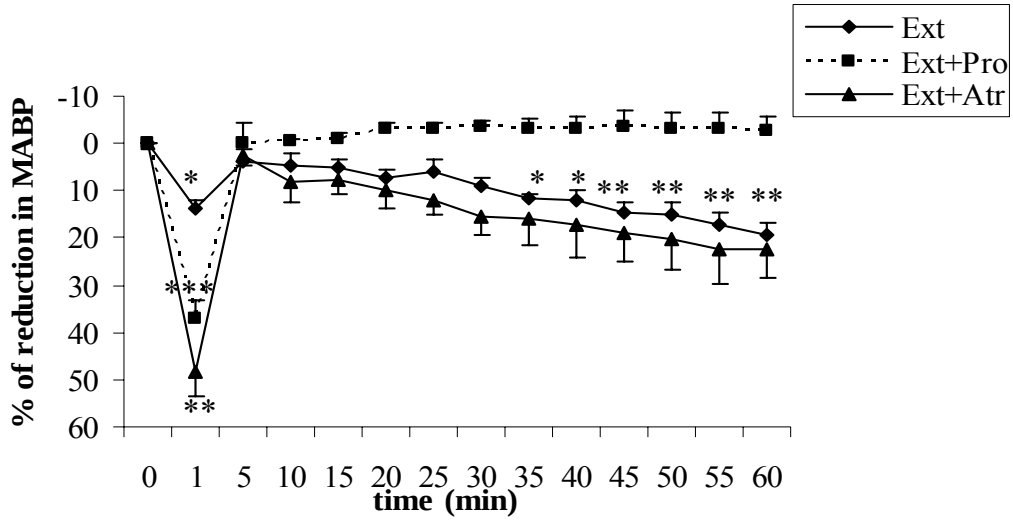

Figure 3: Effect of the ethanol extract of *C. zeylanicum* (Ext) on mean arterial blood pressure (MABP) of normotensive rats and of normotensive rats pre-treated with propranolol (Pro) and/or atropine (Atr). Each point represents the mean ± SEM (n=5)
*p< 0.05, **p< 0.01 and ***p<0.001 significantly different compared to the initial value.

Effects of ethanol extract on isolated aortic strips

Cumulative addition of the extract (1-5 mg/ml) to the bath solution caused concentration-dependent relaxations of the arterial strips precontracted with KCl (60 mM). The relaxation of intact aorta ring segment increased with increasing doses of *C. zeylanicum* bark extract, from 28% at the dose of 1 mg/ml to 74% at the dose of 5 mg/ml. The EC_{50} value for this effect was 1.94 mg/ml (table 1).

Table 1: Effect of cumulative concentrations of *C. zeylanicum* on the aorta precontracted with KCl (60 mM).

[ ]° (mg/ml)	% of Relaxation	CE_{50} (mg/ml)
1	28.05 ± 2.04	1,94
2	51.36 ± 3.67	
3	61.18 ± 3.76	
4	68.03 ± 2.56	
5	74.04 ± 2.20	

Results are expressed as mean ± SEM. Mean values are for five experiments.

When aortic ring segment with or without endothelium was precontracted with KCl (60 mg/ml), a highest dose of the extract (5 mg/ml) produced 94% and 80% relaxation, respectively. The incubation of the plant extract at a concentration of 5 mg/ml resulted in a reduction of the aorta resting tone by 53 mg. Addition of 60 mM of KCl to aortic strips induced a contractile response of 205 mg. Pre-treatment with the extract (5 mg/ml) reduced the amplitude of these contractions (16 mg) by 92% (table 2).

Table 2**:** Effects of the ethanol extract of *C. zeylanicum* (5 mg/ml) on the aortic strip contraction induced by KCl (A) and inhibitory effects of the extract on the contractile responses induced by KCl (B)

	(A) Relaxation (%)		(B) Contractions (mg) induced by KCl (60 mM)		
	+Endo	-Endo	Before C Z	After C Z	% Inhibition
KCl (60 mM)	94.26 ± 4.43	80.82± 0.51	205.87 ± 4.15	16.00 ± 2.66	92.24 ± 1.74

Results are expressed as mean ± SEM. Mean values are for five experiments. +Endo, with endothelium; -Endo, without endothelium; C Z, *Cinnamomum zeylanicum* extract.

Discussion

Blood pressure measurements reflects the status of the cardiovascular system (6), and the maintenance of an adequate blood pressure in the aorta depends on the product of two factors, the cardiac output and the total peripheral resistance of the vessels (7). Therefore, the present study was to evaluate the effects of the ethanol extract from the *Cinnamomum zeylanicum* bark on blood pressure and the aortic vessel. The data on anaesthetized normotensive rats (NTR) and salts loaded

hypertensive rats (SLHR) showed that the intravenous injection of the extract induced a biphasic fall in blood pressure. The first phase that occurred immediately after the administration of the plant extract might be partially attributed to the action on cardiac pump. The first hypotensive response induced by *C. zeylanicum* extract was followed by a rapid return of the blood pressure to the initial level, suggesting once more the hypothesis of the plant extract working on the heart. The second phase occurred slowly and progressively suggesting that it might be due to the reduction of peripheral resistance (5)

The results from the pre-treatment of the rat with propranolol or atropine suggested that the hypotensive properties of the extract are probably due to some interference with the sympathetic and the cholinergic transmission. This is suggested because propranolol enhanced the first hypotensive response of the extract. It is thus possible that this is due to a synergistic bradycardiac effect. Also, it suppressed the second hypotensive response of the extract. It can be argued that *C. zeylanicum* exerts its vasodilation action through β_2 –adrenoceptor mostly located on the wall of vascular smooth muscle (8). Moreover, it is likely that a decrease in β –adrenoceptor activity would result in potentiation of vasoconstriction by unopposed α-adrenoceptor stimulation. In fact, it has been demonstrated in different veins from normotensive rats (9) and on the femoral artery smooth muscle in spontaneously hypertensive rats (10) that the antagonism of the β_2–receptor enhanced the contraction induced by norepinephrine. So, if antagonism of the ethanol extract is capable of provoking the stimulation of α-adrenoceptor, the blockade of these receptors would bring out more details on the partial inhibition of the plant extract by propranolol.

In rats pre-treated with atropine, both the first and the second hypotensive responses of the extract were potentiated. It is known that the parasympathetic nervous system plays a significant role in the control of cardiac activity and arterial blood pressure (11). According to Dhein *et al* (12), the mammalian heart is thought to possess predominantly M_2 muscarinic receptors, as confirmed by the localization of M_2 mRNA in rat heart by in situ hybridisation, the expression of M_1 M_3 and M_4 muscarinic receptor genes have also been reported. However, the role of these gene products and/or their presence has not yet been associated with any functional responses in atria. Since it is established that M_2 –muscarinic receptors are known to be expressed in the heart, the cholinergic stimulation on this organ decreases the role of the spontaneous diastolic depolarisation as well as increasing the repolarising current at the SA node (13), suggesting that, the effect of the extract on the first hypotensive response is also potentiated by a synergistic action on the heart by decreasing the cardiac rate through the stimulation of the M_2 –muscarinic receptors. Since the second hypotensive effect of the extract was enhanced by atropine, this suggests that the extract might be the partial antagonist of atropine and might act at a different site. Taking this in to account, the

sustained hypotensive effect of the extract is probably due to a decrease in total peripheral resistance, probably through none selective stimulation of the muscarinic receptors (M_3 subtype). It is well established that the stimulation of M_3- muscarinic receptors present on vascular beds, produces intense dilation, via release of relaxing factors such as nitric oxide from endothelial cells, despite the lack of apparent cholinergic innervations of most blood vessels (14, 15). However, more investigations are needed before any detailed explanations can be given.

In isolated rat aortic rings, Cumulative concentrations of ethanol extract caused concentration- dependent relaxations of KCl-precontracted aorta much more in the strips preparation with the endothelium than in the aorta without the endothelium. The vascular endothelium plays a crucial role in maintaining the tone of blood vessels by releasing endothelium-derived relaxation factors (EDRF), prostacyclins, hyperpolarizing factors and vasoconstrictor factors, such as endothelin (16, 17). The possible mechanism of action here might involve the production of nitric oxide in the endothelium that results in the relaxation of the preparation contracted by KCl. The contractile responses induced by high K^+ in KCl-depolarized smooth muscle fibres are due to the influx of extracellular Ca^{2+} through L-type voltage-sensitive channels (18, 19). Additionally, the extract did not only inhibit the tonic contraction of aortic rings elicited by 60 mM KCl, but also reduced the resting tone of the vascular smooth muscle. It is known that the resting tone of the vascular smooth muscle can be abolished by removal of extracellular Ca^{2+} or by blockade of voltage dependent calcium channels (20, 21, and 22). Thus, these findings clearly suggest that compounds such as flavonoids and saponins could inhibit calcium through voltage sensitive Ca^{2+} channels in aortic smooth muscle cells (23). It is therefore possible that these same groups of components might be responsible for the hypotensive effects produced by the extract (24, 25).

In conclusion, the results from this study show that the ethanol extract of *C. zeylanicum* is effective in producing hypotensive responses in NTR and SLHR. This hypotensive activity appears in two successive phases. The first phase might be through the effect of the extract on the cardiac pump efficiency, while the second phase might be due to M_3-muscarinic receptors stimulation and vasodilatation. This vasodilatation might be partially through Ca^{2+} channels blocking properties.

Acknowledgments

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