EFFECTS OF *BRILLANTAISIA NITENS* LINDAU (ACANTHACEAE) METHYLENE CHLORIDE/METHANOL LEAF EXTRACT ON RAT ARTERIAL BLOOD PRESSURE AND HEART RATE

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Summary

Brillantaisia nitens Lindau (Acanthaceae) is traditionally used in Cameroon for the treatment of many diseases including cardiovascular disorders. We have studied the effect of the methylene chloride/methanol (CH_2Cl_2/CH_3OH) leaf extracts of *B. nitens* on rat arterial blood pressure and heart rate in normotensive *wistar* rats (NTR), salt laoded hypertensive rats (SLHR), DOCA-salt hypertensive rats (DSHR) and L-NAME hypertensive rats (LNHR), using the direct cannulation method. Intravenous (i.v.) administration of the plant extract (5-80 mg/kg) resulted in a biphasic dose-related hypotensive effect. At the dose of 10 mg/kg, the extract produced a rapid decrease of systolic blood pressure (SBP) by 14%, 16%, 14% and 23% in NTR, SLHR, DSHR and LNHR, respectiveley. In the same animal models, the dose of 40 mg/kg caused decreases of 39%, 26%, 20%, and 34%, respectively. The immediate fall of SBP induced by the plant

extract was followed at higher doses by a sustained antihypertensive response. The most remarkable antihypertensive effect of the plant extract (40 mg/kg) was up to 31%, 60 min after i.v. injection in LNHR. *B. nitens* extract (40 mg/kg) produced a significant reduction of the heart rate by 13% and 29% in NTR and DSHR, respectively. The initial hypotensive effect induced by the plant extract was partially inhibited by atropine and reserpine while the late fall in blood pressure was partially reduced by propranolol and reserpine. These results suggest that the rapid and the late hypotensive activity of *B. nitens* might be due, respectively, to its depressive effect on the cardiac pump and to its vasorelaxant effect.

Keywords: *Brillantaisia nitens*, Rat, Hypotensive effect, Heart rate.

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Introduction

Brillantaisia nitens Lindau (Acanthaceae) is an herbaceous shrub of about 1.5 m high found in Central and West Africa. It is widely used in African traditional medicine to treat skin infections and pain like toothache (1; 2). Some other species have been shown to possess antinociceptive effects (3) or are traditionaly use for their antihypertensive activity (2). In cameroon, decoction of *B. nitens* dried leaves is used by traditional healers of Centre Province, for the management of cardiovascular diseases, especially hypertension. The majority of cameroonian people are hypertensive in rural and urban areas with ages ranging from 25-74 years. The prevalence in men and women is 18.7-23.8% and 12.7–18.8%, respectively (4). In this developing country, the cost of modern drug therapy is prohibitive and as such, many patients resort to traditional herbal medicine for treatment (5; 6). Scientific validation and rationalisation of the medicinal plants need intensification. In previous reports the relaxant effects of *B. nitens* extracts on rat vascular smooth muscle have been demonstrated (7).

The aim of the present study was to evaluate the effect of the dried methylene chloride/methanol extract of *B. nitens* on arterial blood pressure and heart rate in normotensive rat (NTR), salt-loaded hypertensive rats (SLHR), Doca-salt hypertensive rats (DSHR), and L-NAME hypertensive rats (LNHR).

Materials and methods

1. Plant material and extracts

Fresh leaves of *B. nitens* were collected around Yaounde, centre province of Cameroon, in April. The plant material was identified at the National Herbarium in Yaounde, where a voucher specimen no HNC/22729 has been deposited.

The leaves were sun-dried and ground into a powder. Air – dried material (1 kg) was macerated in 7 L of methylene chloride/methanol (v/v) for 48 hours. The solution obtained after filtration was concentrated in a rotary evaporator under reduced pressure to obtain a semi-solid material.

The viscous residues thus obtained was kept at room temperature for one week to obtain 170 g of a completely dried solid mass. The extract was diluted in 2% Tween 20 for subsequent use. 800 mg of this extract was dissolved in 0.2 mL of Tween 20 and the volume of solution adjusted to 10 mL with distilled water to obtain a final extract concentration of 80 mg/mL. Dilution was later made so that all animals received the same volume of solution (0.1 mL/100g body weight). The effect of solvent (2% Tween 20) were tested in order to ascertain that the results obtained were exclusively due to the extract.

2. Phytochemical screening

Phytochemical properties of the CH_2Cl_2/CH_3OH extract were analysed based on Asongalem et al. (8). Chemical groups tested were akaloids, saponins, flavonoids, cardiac glycosides, anthraquinones, phenols and triterpenes (using acetic anhydride and sulphuric acid).

3. Animals

Wistar rats of 8-12 weeks old, for either sex, weighing between 180-230 g were used. The animals were maintained on a 12-hours day/night cycle, with free access to standard laboratory rat chow and tap water. Normotensive rat (NTR) were used to evaluate effects of the plant extract on arterial blood pressure, heart rate and its mechanism of action. To determine the antihypertensive activity mechanism of *B. nitens*, salt-loaded hypertensive rats (SLHR), Doca-salt hypertensive rats (DSHR), and L-NAME hypertensive rats (LNHR) were used for blood pressure and heart rate studies. SLHR were obtained from NTR by given orally, once a day, a 18% NaCl solution (1 mL/100 g body weight) with free access to 1.2% NaCl as drinking water (6). DSHR were obtained from NTR, without nephrectomy, as described by Vogel and Vogel (9). The rats were injected twice weekly with desoxycorticosterone-acetate (20 mg/kg, s.c.) in carboxymethyl-cellulose (5% of DOCA weight), for 4 weeks. Drinking water was replaced with a 1% NaCl solution. To obtain LNHR, NTR were given by gastric intubation Nitro-L-arginine methyl ester (L-NAME, 40 mg/kg daily at 1 mL/100 g body weight) for 4 weeks. At the end of the respective treatment, rats showing a systolic blood pressure higher than 150 mmHg were considered as hypertensive.

4. Blood pressure and heart rate measurements

The rats were anaesthetized using an intraperitoneal injection of urethane (1 g/kg). The trachea was exposed and cannulated to facilitate spontaneous respiration. The arterial blood pressure was measured from right carotid artery via an arterial cannula connected to a pressure transducer, coupled with a hemodynamic recorder Biopac Student Lab. (MP35) and computer. The heart rate was also monitored. The animals were allowed to stabilize for at least 30 min before administration of any test substances. The plant extract was injected via a cannula inserted into the left femoral vein. The effects of the plant extract were compared with those of acetylcholine (10 µg/kg) and isoprenaline (10 µg/kg). The dose of 40 mg/kg was examined after administration of atropine (1 mg/kg) and propranolol (100 µg/kg). Atropine and propranolol were injected intravenously, 5 min before administration of the plant extract. β-adrenoceptor was established by its specific bloker, propranolol. The effectiveness of blockade was tested by injecting 10 µg/kg of isoprenaline (agonist). In another set of experiment, reserpine (5 mg/kg) was given orally to NTR, once a day for three days and the effects of the extract on arterial blood pressure and heart rate evaluated. Blood pressure and heart rate were observed for 1 h after test drug administration. Changes in blood pressure and heart rate were expressed in real values, or as a percentage of the control values obtained just before the administration of test substances.

5. Drugs

Urethane, isoprenaline and acetylcholine chloride were obtained from Prolabo, France. Atropine sulphate, propranolol, and L-NAME from Sigma Chemical, St Louis, MO, USA. Heparine was from Sanofi, France. The drugs were freshly prepared before the experiment. All drugs were dissolved in distilled water except for the plant extract that was dissolved in 2% Tween 20 and the solution adjusted with distilled water.

6. Statistical analysis

Data were shown as mean ± S.E.M. Statistical significance was estimated by one way ANOVA test. A P-value less than 0.05 was regarded to be significant.

Results

1) Phytochemical analysis

Phytochemical analysis revealed the presence of alkaloïds and saponins in the CH_2Cl_2/CH_3OH extract. Flavonoids, cardiac glycosides, anthraquinones, phenols and triterpenes were absent

2) Effects of the methylene chloride/methanol extract of B. nitens *on normotensive rats arterial blood pressure*

In normotensive anesthetized rats (NTR) intravenous (i.v.) injection of *B. nitens* extract (5, 10, 20, 40 and 80 mg/kg) produced rapid and dose-dependent decreases of blood pressure. Systolic blood pressure (SBP) dropped from 107.82 ± 3.82 mm Hg to 93.70 ± 5.12 mmHg at the lower dose and from 122.22 ± 3.15 mmHg to 75.04 ± 1.44 mmHg at the higher dose, representing decreases of 13% and 39%, respectiveley.

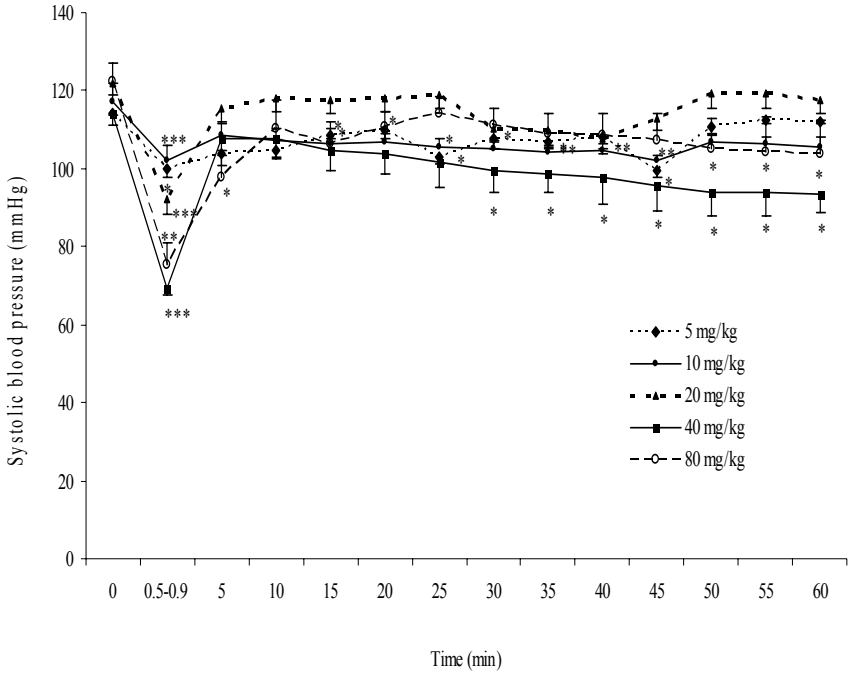

Figure 1: Effects of *B. nitens* methylene chloride/methanol extract on the systolic blood pressure in normotensive rats. Each point represents the mean ± SEM of 5 animals. *p<0.05, **p<0.01 and ***p<0.001. Significantly different from initial value.

The rapid fall in blood pressure was followed in all doses by a rise of arterial blood pressure which tends to return to its initial value. At the dose of 40 mg/kg, the maximal rapid decrease of 39% was followed by an significant fall (18%, $p<0.05$) after 60 min. Figure 1 shows the time course of the hypotensive action of *B. nitens* CH_2Cl_2/CH_3OH extract at doses of 5-80 mg/kg. By comparison, acetylcholine (10 µg/kg), isoprenaline (10 µg/kg) and the plant extract (40 mg/kg) induced a transient fall of 34.94% ± 3.63%, 24.74% ± 3.03% and 39.00 ± 2.41% in SBP. The initial effect of *B. nitens*, in contrast of that of acetylcholine and isoprenaline, was followed by a sustained hypotensive effect which was maintained for one hour (Fig. 2).

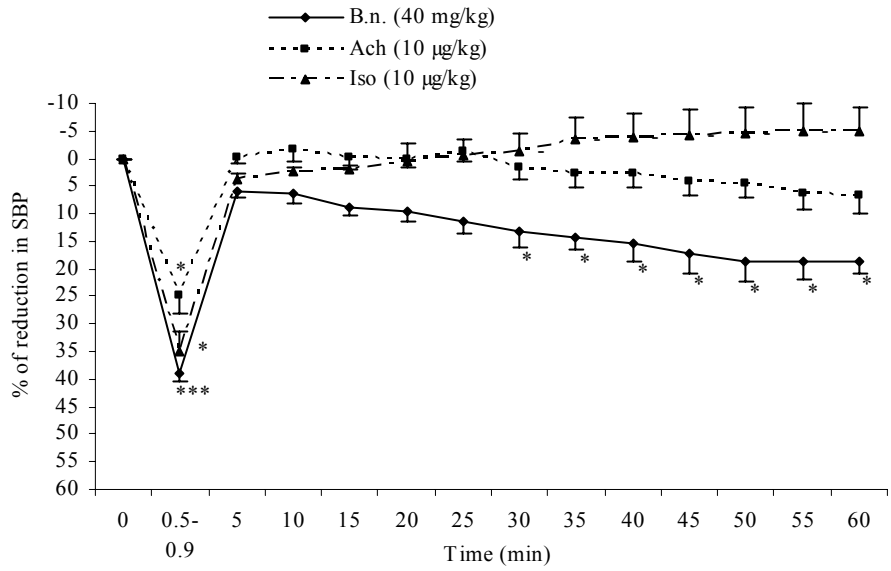

Figure 2: Effects of *B. nitens* methylene chloride/methanol extract (B.n.), isoprenaline (Iso) and acetylcholine (Ach) on the systolic bood pressure in normotensive rats. Each point represents the mean ± SEM of 5 animals. *$p<0.05$ and ***$p<0.001$. Significantly different from initial value.

3) Effects of the pharmacological antagonists on normotensive rats arterial blood pressure

The effects of three antagonists (atropine, propranolol and reserpine) on the hypotensive action of *B.nitens* (40 mg/kg) were investigated in the mean arterial blood pressure (MBP) of NTR. The plant extract was injected 5 min after the administration of atropine (1 mg/kg) or propranolol (100 µg/kg). It was found that atropine significantly ($p<0.05$) inhibited by 43.69% the initial fall of arterial blood pressure induced by *B. nitens* (40 mg/kg) without significant effect on the late hypotensive response. Propranolol had no significant effect on transient hypotensive effect of the plant extract but was able to inhibit completely the late hypotensive activity. In rats pre-treated with reserpine, the first and the second hypotensive responses provoked by the plant extract was significantly inhibited by 51.24% and 99.93%, respectively (Fig. 3).

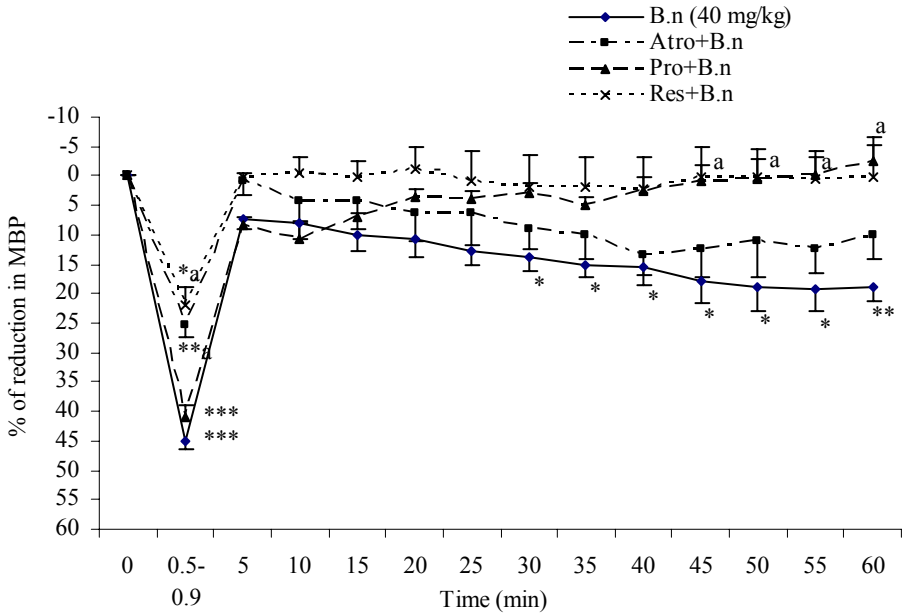

Figure 3: Effects of *B.nitens* methylene chloride/methanol extract (40 mg/kg) on the mean blood pressure (MBP) of normotensive rats and of normotensive rats pre-treated with atropine (Atro), propranolol (Pro) and reserpine (Res). Each point represents the mean ± SEM of 5 animals. *$p<0.05$, **$p<0.01$ and ***$p<0.001$. Significantly different from initial value. $^{a}p < 0.05$. Significantly different from *B. nitens* effects in normotensive rats.

4) Effects of the methylene chloride/methanol extract of B. nitens *on hypertensive rats arterial blood pressure*

In salt-loaded hypertensive rats (SLHR) and DOCA-Salt hypertensive rats (DSHR), the leaf CH_2Cl_2/CH_3OH extract of *B. nitens* (10-40 mg/kg) induced a significant and dose-dependent fall in SBP, immediatly after i.v. administration. The immediat fall in arterial blood pressure induced by the plant extract was followed in all doses by a non significant second antihypertensive response in SLHR (data not shown). As shown in Figure 4, the plant extract (40 mg/kg) caused rapid decreases of SBP by 26.00% and 20.44% in SLHR and DSHR, respectively. The second hypotensive effect started 10-15 min after i.v. injection and was up to 5.28% ($p>0.05$) and 9.85% ($p < 0.05$), at 60 min, respectively in SLHR and DSHR, as compared to the initial value.

In L-NAME hypertensive rats (LNHR), *B. nitens* extract induced a significant dose-dependent biphasic fall in arterial blood pressure. The SBP decreased from 181.00 ± 5.00 to 119.00 ± 3.00 mmHg immediately after administration of the extract (40 mg/kg), giving a fall of 33.97% ± 2.38%. The second phase started when the blood pressure had not completely recovered and reached a peak of 30.86% ± 4.10% fall after 60 min (Fig. 4).

5) Effects of the methylene chloride/methanol extract of B. nitens *on normotensive rats heart rate*

As shown in Table 1, *B. nitens* at the lower doses (10 mg/kg and 20 mg/kg) produced no significant change in the normotensive rats heart rate. At the higher doses, the plant extract induced significant changes immediatly after i.v. injection. The decrease in heart rate was 13.41% (from 335.60 ± 10.85 to 290.58 ± 5.95 beats/min) at the dose of 40 mg/kg and 22.80% (from 331.54 ± 8.85 to 255.97 ± 15.95 beats/min) at the dose of 80 mg/kg.

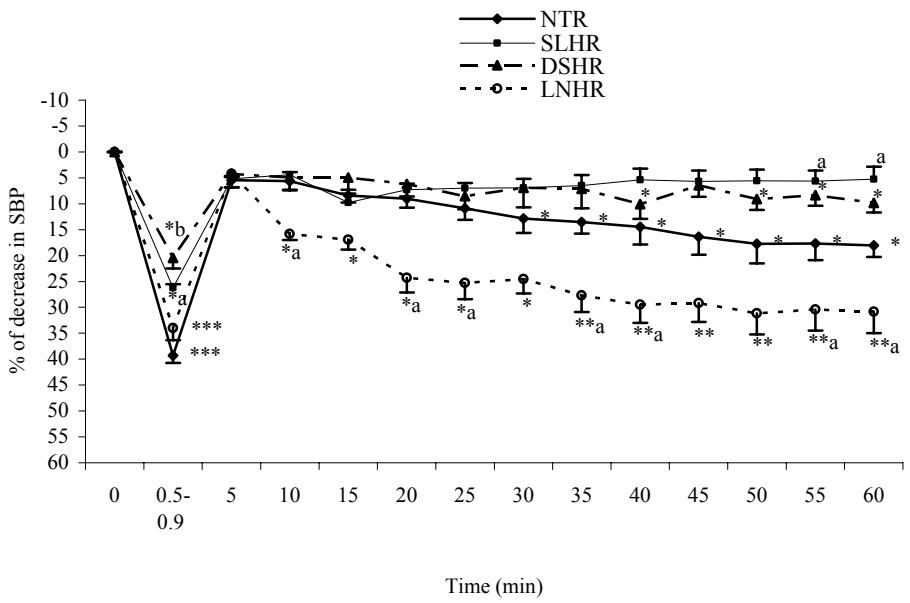

Figure 4: Comparative effects of *B. nitens* methylene chloride/methanol extract (40 mg/kg) on the systolic blood pressure in normotensive rats and hypertensive rats. Each point represents the mean ± SEM of 5 animals. *p<0.05, **p<0.01 and ***p<0.001. Significantly different from initial value. [a]p<0.05 and [b]p<0.01. Significantly different from NTR.

Table 1: Effects of *B. nitens* methylene chloride/methanol extract on the heart rate in anaesthetized normotensive rats

Time (s)	**Heart rate (beats/min)**			
	10 mg/kg	**20 mg/kg**	**40 mg/kg**	**80 mg/kg**
0	344±8	341±10	336±11	332±9
5-20	340±10	335±12	291±6 [b]	256±16 [a]
300	352±10	345±10	316±7	261±19
600	349±19	345±6	316±10	264±15
900	333±12	348±13	313±5	300±12
1200	336±12	357±14	319±5	314±12
1500	337±17	350±10	322±15	323±10
1800	360±18	345±11	301±24	332±18

Each value represents the mean ± SEM of 5 animals. [a]P<0.05, [b]P<0.01. Significantly different from initial value

6) Effects of pharmacological substances on normotensive rats heart rate

Acetylcholine (10 µg/kg), like the plant extract (40 mg/kg) produced immediately after administration a significant fall in heart rate, while isoprenaline (10 µg/kg) caused a significant increase in normotensive rat heart rate. Ach caused a decrease from 355 ± 16 beats/min to 301 ± 12 beats/min, corresponding to 15.77% ($p<0.05$) fall, while isoprenaline caused an increase from 363 ± 7 to 404 ± 2 beats/min, corresponding to an increase of 11.26% ($p<0.01$). The effect of *B. nitens* (40 mg/kg) was not inhibited by propranolol (100 µg/mg), which totaly inhibited the increasing effect of isoprenaline on heart rate (data not shown). In rats pre-treated with reserpine (5 mg/kg/day) and atropine (100 µg/kg), the fall in heart rate due to the extract (40 mg/kg) was inhibited (Table 2).

7) Effects of B. nitens on salt-loaded hypertensive rats (SLHR), DOCA-Salt hypertensive rats (DSHR) and L-NAME hypertensive rats (LNHR)

In hypertensive rats (SLHR, DSHR and LNHR), the plant extract, only at the dose of 40 mg/kg and in DSHR, produced a significant ($P<0.05$) fall of 28.69% in heart rate (Data not shown).

Table 2: Effect of *B. nitens* methylene chloride/methanol extract (40 mg/kg) on the heart rate in normotensive rats pre-treated with atropine (Atro), propranolol (Pro) and reserpine (Res)

	Heart rate (beats/min)					
Time (s)	**Bn (40 g/kg)**	**Res+Bn**	**Iso (n=4)**	**Prop+Bn**	**Ach.(n=4)**	**Atro+Bn**
0	336 ± 11	346 ± 16	363 ± 71	305 ± 7	355 ± 16	354 ± 13
5-20	291 ± 6 [b]	340 ± 15	404 ± 2 [b]	286 ± 3 [a]	301± 13 [a]	340 ± 9
300	316 ± 7	343 ± 16	381 ± 3	288 ± 10	352 ± 14	339 ± 9
600	316 ± 10	342 ± 16	368 ± 7	294 ± 8	349 ± 25	342 ± 9
900	313 ± 5	341 ± 15	354 ± 19	305 ± 10	344 ± 18	339 ± 8
1200	319 ± 5	342 ± 14	357 ± 19	315 ± 12	347 ± 15	337 ± 7
1500	322 ± 15	342 ± 14	355 ± 20	320 ± 15	340 ± 16	325 ± 8
1800	301 ± 24	340 ± 13	357 ± 21	326 ± 17	344 ± 16	328 ± 15

Each value represents the mean ± SEM of 5 animals. [a]$P<0.05$ and [b]$P<0.01$. Significantly different from initial value. Iso: isoprenaline; Ach: acetylcholine; B n: *B. nitens* methylene chloride/methanol extract

Discussion

The present results indicate that CH_2Cl_2/CH_3OH leaves extracts of *B. nitens* induced significant, dose-dependent and biphasic fall of arterial blood pressure in normotensive rats, DOCA-Salt hypertensive rats and L-NAME hypertensive rats.

In normotensive rats, the CH_2Cl_2/CH_3OH extract of *B. nitens*, like acetylcholine, induced a rapid fall of arterial pressure, suggesting an action on cardiac pump. The highest dose of 80 mg/kg produced a 39% decrease of the SBP, but seems to be toxic by i.v. route. In fact, we observed a 28.57% mortality with that dose. The transient hypotensive effect provoked by the plant extract was accompanied, in the higher dose of 40 mg/kg, with a significant decrease of the heart rate. Cardiac activity was not significantly affected by lower doses of the plant extract. This could be explained by the fact that the first hypotensive activity of the extract is not only due to its effect on cardiac efficiency. Similar effects were obtained with some alkaloïds (10; 11). Our phytochemical analysis revealed the presence of alkaloïds in the CH_2Cl_2/CH_3OH extract of *B. nitens*, thus the effect of *B. nitens* could be due to the presence of alkaloïds.

The initial hypotensive effect of the plant extract was followed by a sustained hypotensive activity which was stabilized at lower pressure levels when compared with the initial pressure value. Similar results were obtained by Dimo et al. (12), studying the effect of the neutral extract of *Bidens pilosa* on rat cardiovascular system. The sustained fall of pressure was maintained for more than one hour. The sustained hypotensive effect of the extract could be due to its vasorelaxant properties. In fact, Dimo et al. (7) while working on isolated rat vascular smooth muscle showed the vasorelaxant effect of the CH_2Cl_2/CH_3OH extract of *B. nitens*. In our study, isoprenaline (10 µg/kg) also produced an immediat fall of blood pessure, which was completly suppressed by propranolol (100 µg/kg). The late hypotensive effect of *B. nitens* (40 mg/kg) was inhibited by propranolol (100 µg/kg), a β-adrenergic antagonist (12; 13; 14). The fact that antihypertensive effect of *B. nitens* was significantly inhibited by propranolol suggest that extract exerts its sustained hypotensive effect through β-adrenergic vasodilation. The negative chronotropic effect of the extract was not inhibited by propranolol (100 µg/kg). This result shows that *B. nitens* may not act as a cardiac β-adrenergic blocker. Similar results were reported by Eno and Owo

(14) in their study on the cardiovascular effects of an *Elaeapphorbia drupifera* roots extract. In contrast, reserpine (5 mg/kg) inhibited partialy the first hypotensive effect and the negative chronotropic effect of the extract. These results suggest a partial action of extract by blocking cardiac α_1-adrenergic receptors. The extract could inhibit cardiac and vascular α_1-adrenoceptors, and then such a mechanism may be associated to the direct calcium channels inhibition (7).

Our results demonstrated that *B. nitens* extract also produced a dose-dependent immediate decrease of blood pressure in SLHR, DSHR and LNHR. The excessive consumption of NaCl by rats provokes, as in Human, an arterial hypertension (6; 15; 16). In SLHR, the peripheral resistances are progressively increased by the augmentation of intracellular concentration of Ca^{2+} in vascular muscle cells (6; 17; 18). The fact that extracellular Na^+ concentration increased could cause the inactivation K_{ATP} channels or Na/Ka pump, giving rise to depolarization and calcium influx in vascular muscle cells (19). The hypotensive effect of *B. nitens* may be due to an activation of K_{ATP} channels (7). Similar results were reported by Wu et al. (20).

Increased sympathetic activity has been found to be associated with high sodium intake in experimental animal with deoxycorticosterone acetate-salt hypertension (21). DOCA is a mineralocorticoïd. Mineralocorticoïds like aldosterone, increase K^+ mobilisation and Na^+ reabsoption in renal tubes (13; 15). Thus, the association of DOCA and NaCl increase natremia, volemia, and secondary peripheral resistances (16). The vascular tone increases and then, induces hypertension. The low sustained hypotensive effect of the plant extract, in this model, could be due to the highest vascular tone.

Intravenous injection of the plant extract provoked a biphasic fall of blood pressure in LNHR. The sustained hypertensive activity of *B. nitens* was more pronounced in LNHR as compared to NTR and SLHR. It has been reported that the high blood pressure in those hypertensive rats is a direct consequence of the decrease or the suppression of NO synthesis by vascular endothelial cells. In fact, L-NAME (Nitro-L-arginine methyl ester) is a non selective NO synthase inhibitor (16; 22). Its chronic administration to rats provokes an increase of arterial blood pressure by NO concentration decrease. Our results show that the plant extract was more potent

during the second phase of hypotensive activity which correspond to the action of the plant extract on the vascular resistance. These observations suggest a direct vasorelaxant action of *B. nitens*, which can play an important role in hypotensive effect.

In conclusion, the CH_2Cl_2/CH_3OH extract from *B. nitens* leaves is effective in producing hypotensive responses in NTR, SLHR, DSHR and LNHR. The hypotensive activity appears in two successive phases. The first hypotensive phase is due, at least partially, to the negative chronotropic effect and the second to the action of the extract on the vascular resistance. These effects are mediated trough α_1-adrenoceptors blockade. More work is underway to elucidate the real mechanism of action.

Acknowledgements

This work was supported by a research grant (N° 03-460/RG/BIO/AF/AC) from the Third World Academy of Science.

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