

Full Length Research Paper

# Cardiodepression as a possible mechanism of the hypotensive effects of the methylene chloride/methanol leaf extract of *Brillantaisia nitens* Lindau (Acanthaceae) in rats

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*Brillantaisia nitens* Lindau (Acanthaceae) is traditionally used in Cameroon for the management of many diseases including cardiovascular disorders. The aim of this study was to demonstrate the contribution of cardiodepressive activity of methylene chloride/methanol leaf extract of *B. nitens* to its hypotensive action in normotensive (NTR) and deoxycorticosterone acetate-salt hypertensive rats (DSHR). In this study, we used the direct cannulation method for blood pressure measurements and electrodes for electrocardiogram (ECG). In NTR, the systolic blood pressure dropped by 12.6, 13.8, 22.5 and 39.3% at the doses 5, 10, 20 and 40 mg/kg, respectively. In DSHR, systolic blood pressure decreased by 13.8, 16.2, 16.3 and 20.4% at the same doses, respectively. *B. nitens* extract (40 mg/kg) produced a significant reduction of the heart activity while the blood pressure rapidly dropped. At this same dose in NTR, *B. nitens* induced a negative chronotropic effect by causing a 20.59% ( $p < 0.05$ ) R-R interval elongation. More also, On P wave and T wave magnitude, the plant extract at the same dose induced a significant ( $p < 0.05$ ) decrease of 21.05 and 15.79%, respectively. For DSHR, *B. nitens* extract (40 mg/kg) induced significant changes on T wave duration, R-R interval and P wave magnitude. *B. nitens* extract decreased T wave duration by 44.44% ( $p < 0.05$ ), increased R-R interval by 100% ( $p < 0.01$ ) and decreased P wave magnitude by 20% ( $p < 0.05$ ). These results confirm our previous findings that the immediate hypotensive effect of *B. nitens* is partly due to its depressive effect on the cardiac pump.

**Key words:** *Brillantaisia nitens*, methylene chloride/methanol extract, hypotensive effect, cardiodepressive activity, rat.

## INTRODUCTION

*Brillantaisia nitens* Lindau (Acanthaceae) has been shown to possess several medicinal properties (Burkill, 1985; Adjanohoun et al., 1996; Matheus et al., 2005; Dimo et al., 2007; Akah et al., 2009). The leaves of *B.*

*nitens* have been used for many decades against cardiovascular diseases, especially hypertension (Bopda et al., 2007).

Hypertension is currently increasing in developing countries (Fezeu et al., 2010) such as Cameroon, where the urban area prevalence in 2003 was 25.6 and 23.1% in male and female subjects (Kamadjeu et al., 2006). Since heart rate is one of the factors involved in blood pressure value, it is assumed that the higher the heart

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performance is, the higher the blood pressure would be. In our previous work we reported that a decrease in heart rate contributes to the hypotensive effect of methylene chloride/methanol extract of *B. nitens* in normotensive and hypertensive rats (Bopda et al., 2007).

The aim of this study was to demonstrate the mechanistic contribution of cardiodepressive activity of *B. nitens* extract to its hypotensive activity on normotensive (NTR) and deoxycorticosterone acetate-salt hypertensive rats (DSHR).

## MATERIALS AND METHODS

### Plant material

Fresh leaves of *B. nitens* were collected around Yaounde, Centre Province of Cameroon, in April 2007. The plant material was identified at the National Herbarium in Yaounde, where a voucher specimen no HNC/22729 has been deposited. The leaves were air-dried and ground into powder. Air-dried material (1 kg) was macerated in 7 L of methylene chloride/methanol (v/v) for 48 h and the solution obtained after filtration was then concentrated in a rotary evaporator under reduced pressure to obtain a semi-solid material. The viscous residue thus obtained, was kept at room temperature for one week to obtain 170 g of a completely dried solid mass. The extract (800 mg) was dissolved in 0.2 ml of Tween 20 and the volume of solution was then 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 (1 ml/100 g or 0.1 ml/100 g body weight, respectively for toxicity or electrocardiogram studies).

### Acute toxicity test

50 mice were divided into five groups of ten (sex equal) per group after 12 h fasting period, with free access to water. The mice in one of the groups received Tween 20 solution 2% (1 ml/100 g of body weight, *per os*), while those in the four other groups received oral doses of the extract (1000, 2000, 4000 and 8000 mg/kg, respectively). The animals were observed for obvious toxic symptoms (changes in body weight, aggressiveness, sensitivity to noise and touch, stools aspect and locomotion), and eventual mortality in each group was searched 48 h after extract administration. Animal were kept under observation for 14 days (Joshua et al., 2008; Abdulla et al., 2010).

### Arterial blood pressure and cardiac activity

#### Experimental animals

75 *Wistar* rats of 8 to 12 weeks old for both sex, and weighing between 180 to 230 g were used. They were shared into groups of five as follows: eight groups (four per animal model) were used for blood pressure measurement. For ECG investigations, four of the aforementioned groups were exploited (since blood pressure and ECG values were simultaneously recorded), while seven groups were newly set (reference drugs and their antagonists). They were carefully handled according to guidelines for the care and use of laboratory animals approved by the Japanese Pharmacological

Society. The animals were maintained on a 12-h day/night cycle, with free access to standard laboratory rat chow and tap water. Normotensive rats (NTR) were used to evaluate the effects of the plant extract on blood pressure and ECG and its possible mechanism of action. To better understand the mechanism of action, we used DSHR obtained from uninephrectomized NTR, as described by Vogel and Vogel (1997). The rats were injected twice weekly with deoxycorticosterone acetate (DOCA 20 mg/kg, s.c.) in carboxymethyl-cellulose (5% of DOCA weight), for four weeks. Drinking water was replaced with a 1% NaCl solution. At the end of the treatment, rats showing a systolic blood pressure higher than 150 mmHg were considered as hypertensive.

### Measurement of blood pressure, cardiac activity and evaluation of the effects of the plant extract

The rats were anaesthetized using an intraperitoneal injection of urethane (1 g/kg). The trachea was exposed and cannulated to facilitate spontaneous respiration. Blood pressure was measured by the direct method from right carotid artery, using a cannula connected to a pressure transducer. The latter was coupled with a Biopac Student Lab. (MP35) hemodynamic recorder and a computer. ECG was measured using high sensitivity needles (electrodes), connected to the same recorder and computer. The animals were allowed to stabilize for at least 30 min before the administration of any test substances. The plant extract (5, 10, 20 and 40 mg/kg) was injected via a cannula inserted into the left femoral vein.

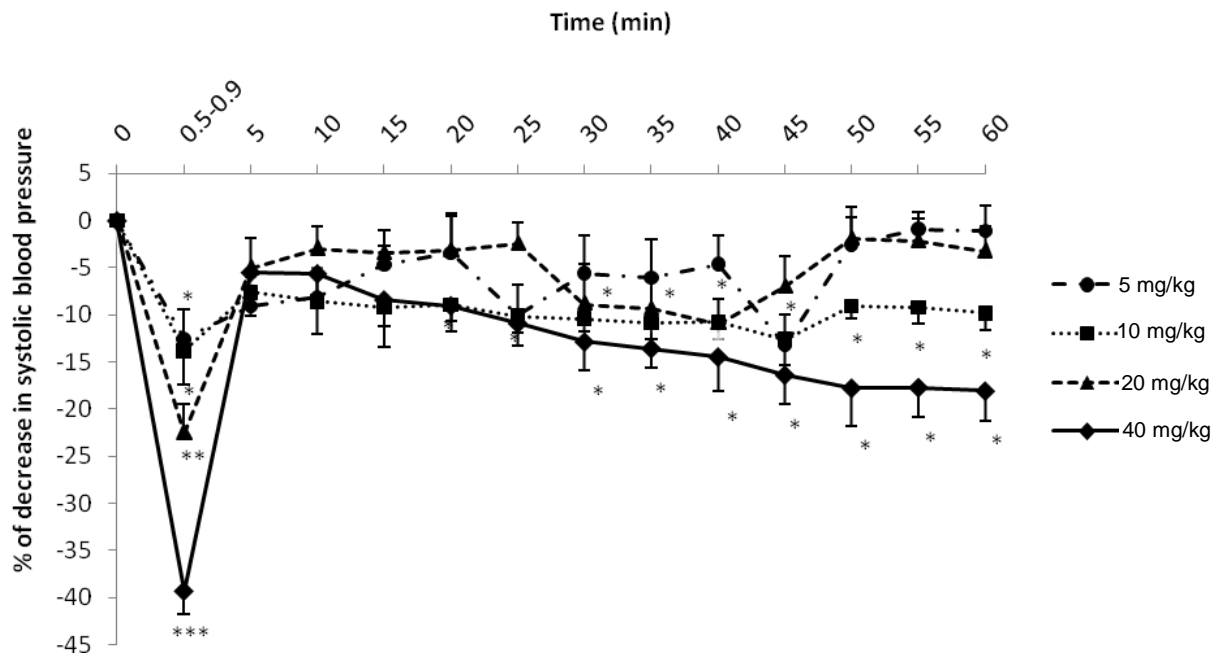
Moreover, the effects of the plant extract were compared with those of acetylcholine and isoprenaline (10 µg/kg each). The effect of the dose 40 mg/kg (which appeared to be the optimal, from our previous investigations) was examined after administration of atropine (1 mg/kg) and propranolol (100 µg/kg). Atropine and propranolol were injected intravenously 5 min before administration of the plant extract. 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. The effects of the extract were evaluated on the blood pressure and ECG parameters, which were observed for 1 h after test drug administration. The effect of solvent (2% Tween 20) was tested in order to ascertain that the results obtained were exclusively due to the extract. Changes in blood pressure and ECG were expressed in real values, or as a percentage of the control values obtained just before the administration of test substances.

### Drugs

Urethane, isoprenaline and acetylcholine chloride were obtained from Prolabo, France, while atropine sulphate, propranolol, DOCA and carboxymethyl-cellulose were obtained from Sigma Chemical, St Louis, MO, USA. Heparin 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.

### Statistical analysis

Data were shown as mean ± S.E.M. Statistical significance was estimated by one way ANOVA, followed by Dunnett's test. Difference between means were regarded as significant at  $p < 0.05$ .



**Figure 1.** Effects of *B. nitens* methylene chloride/methanol extract on the systolic blood pressure in normotensive rats. Each point represents the mean  $\pm$  SEM;  $n = 5$ ; \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$ , significantly different from initial value.

## RESULTS

### Acute toxicity test

Mice administered with *B. nitens* methylene chloride/methanol leaf extract did not develop any clinical sign of toxicity, either immediately or during the post-treatment period, at the doses of 1000, 2000, 4000 and 8000 mg/kg. No mortality was observed after 48 h.

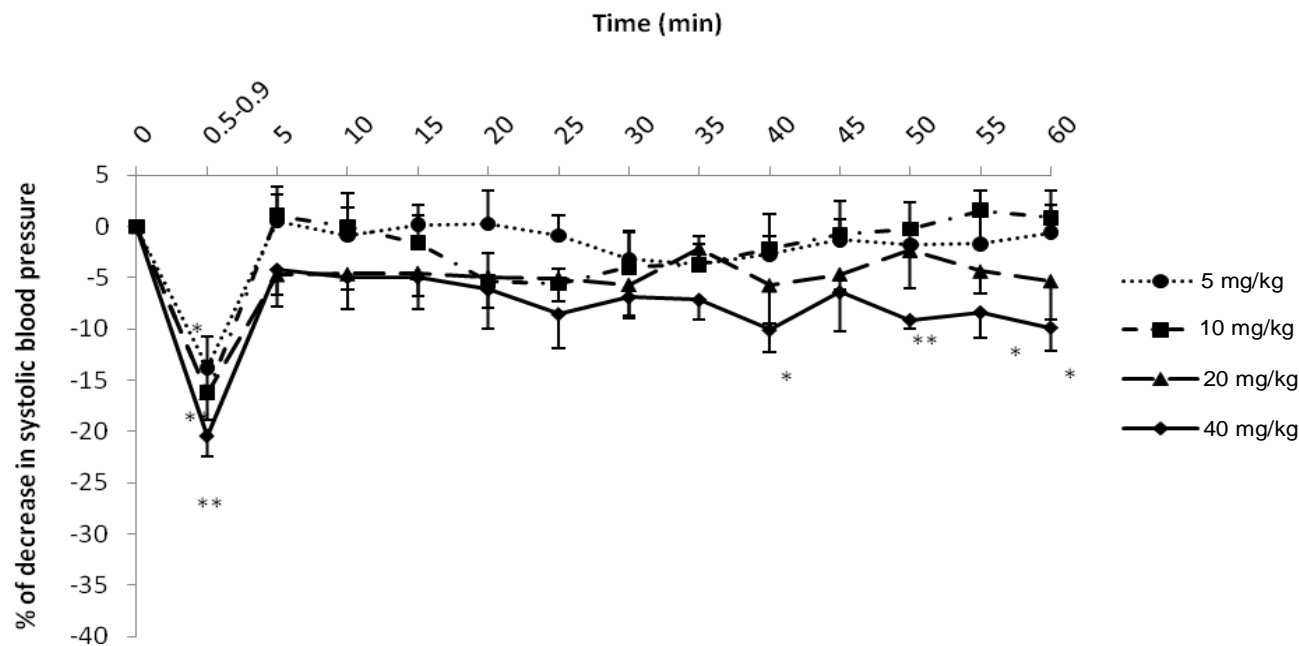
### Effects of the methylene chloride/methanol extract of *B. nitens* on the blood pressure of normotensive and hypertensive rats

It was noticed that injection of the vehicle did not change the blood pressure baseline. The baseline in normotensive rats ranged from  $107.82 \pm 3.82$  to  $114.11 \pm 3.15$  mmHg while in hypertensive rats, the range was  $153.62 \pm 3.82$  to  $168.11 \pm 5.10$  mmHg. Percentages were used since the baseline varies. In normotensive rats (NTR) and DOCA-salt hypertensive rats (DSHR), the  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  leaf extract of *B. nitens* (5 to 40 mg/kg) induced a significant and dose-dependent fall in systolic blood pressure, immediately after i.v. administration. In NTR (Figure 1), systolic blood pressure dropped by 12.6, 13.8, 22.5 and 39.3% at the doses of 5, 10, 20 and 40

mg/kg, respectively while in DSHR (Figure 2), systolic blood pressure decreased by 13.8, 16.2, 16.3 and 20.4% at the same doses, respectively. Hence, in the two animal models, the rapid fall in blood pressure was followed by an increase which tended to return to its initial value and thereafter variable sustained decreases were observed. At the dose of 40 mg/kg, the maximal rapid decreases of 39 (NTR) and 20% (DSHR) were recorded. Also, at the end of 60 min, the systolic blood pressure significantly ( $p < 0.05$ ) fell by 18.1 (NTR) and 9.9% (DSHR).

### Effects of the methylene chloride/methanol extract of *B. nitens* on ECG of normotensive rats

All the variations in ECG parameters occurred within 10 s after drugs administration (Figures 3 and 4). In NTR (Table 1), *B. nitens* (40 mg/kg) induced an elongation of R-R interval from  $170 \pm 10$  to  $205 \pm 10$  min, representing a significant increase of 20.59% ( $p < 0.05$ ). On P wave and T wave magnitude, the plant extract at the same dose induced a drop from  $0.19 \pm 0.03$  to  $0.15 \pm 0.01$  mV and from  $0.19 \pm 0.01$  to  $0.16 \pm 0.01$  mV, representing a significant ( $p < 0.05$ ) decrease of 21.05% and 15.79%, respectively. Furthermore, we noticed a brief and slight fall of the isoelectrical line in some animals, as similar to that in Figures 3A and 4B.

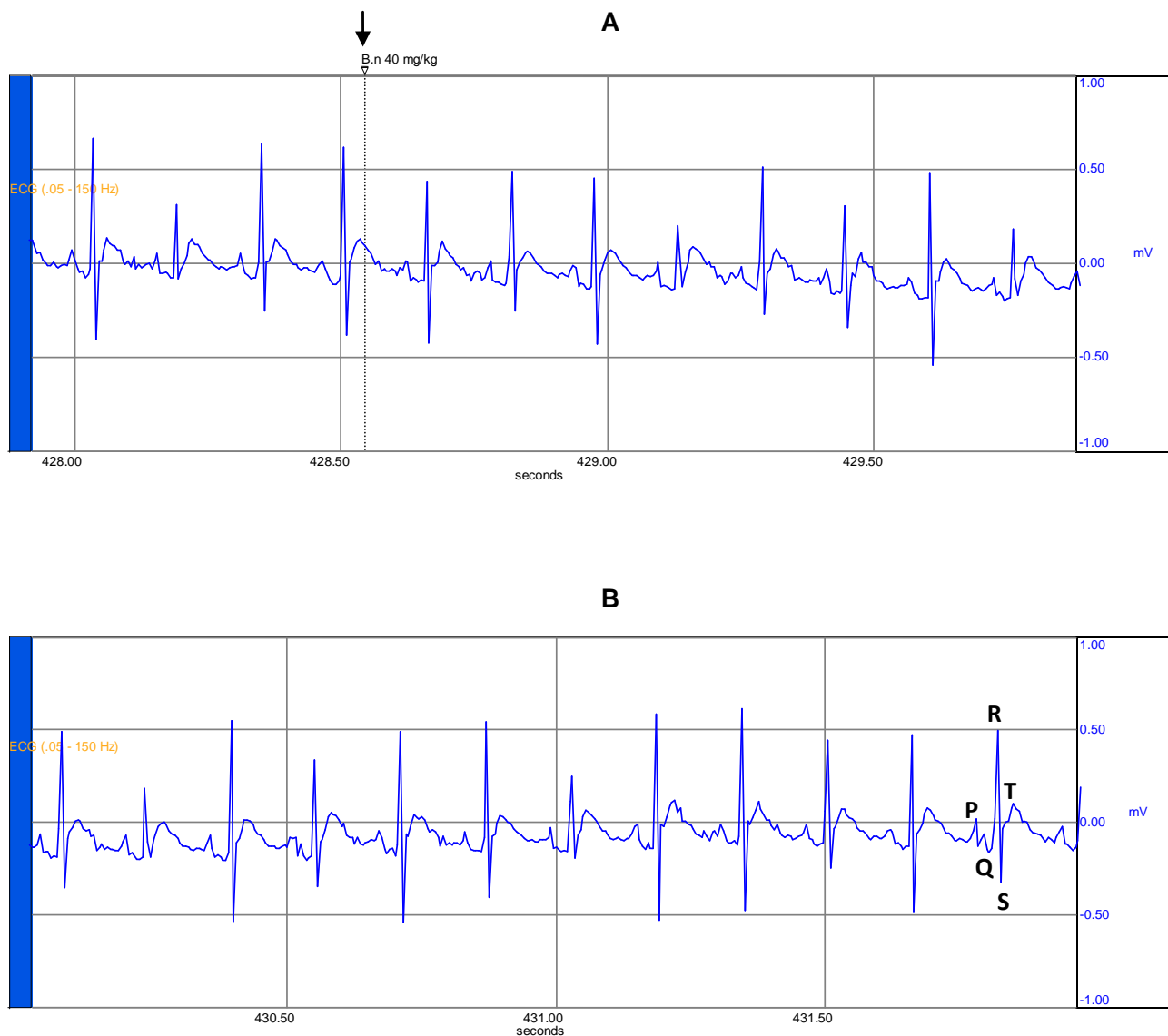


**Figure 2.** Effects of *B. nitens* methylene chloride/methanol extract on the systolic blood pressure in hypertensive rats (DSHR). Each point represents the mean  $\pm$  SEM; n = 5; \*p<0.05 and \*\*p<0.01, significantly different from initial value.

**Table 1.** Effects of *B. nitens* methylene chloride/methanol extract and reference drugs on ECG of normotensive rats.

ECG parameter	Time (min)							Magnitude (mV)		
	P wave	P-R interval	P-R segment	QRS complex	Q-T interval	T wave	R-R interval	P wave	Complex QRS (R)	T wave
Control	30 $\pm$ 8	50 $\pm$ 8	20 $\pm$ 8	30 $\pm$ 6	110 $\pm$ 10	80 $\pm$ 10	170 $\pm$ 10	0.19 $\pm$ 0.03	0.85 $\pm$ 0.04	0.19 $\pm$ 0.01
<i>B. nitens</i> (10 mg/kg)	30 $\pm$ 5	50 $\pm$ 7	20 $\pm$ 7	30 $\pm$ 6	100 $\pm$ 20	70 $\pm$ 10	170 $\pm$ 10	0.19 $\pm$ 0.01	0.83 $\pm$ 0.04	0.17 $\pm$ 0.02
<i>B. nitens</i> (20 mg/kg)	30 $\pm$ 7	50 $\pm$ 7	20 $\pm$ 4	33 $\pm$ 5	105 $\pm$ 20	72 $\pm$ 8	180 $\pm$ 10	0.18 $\pm$ 0.02	0.85 $\pm$ 0.06	0.18 $\pm$ 0.02
<i>B. nitens</i> (40 mg/kg)	30 $\pm$ 4	50 $\pm$ 9	20 $\pm$ 5	30 $\pm$ 5	105 $\pm$ 20	75 $\pm$ 10	205 $\pm$ 10*	0.15 $\pm$ 0.01*	0.84 $\pm$ 0.01	0.16 $\pm$ 0.01*
Acetylcholine (10 $\mu$ g/kg)	30 $\pm$ 7	50 $\pm$ 6	20 $\pm$ 4	30 $\pm$ 6	80 $\pm$ 15	50 $\pm$ 20	240 $\pm$ 20*	0.19 $\pm$ 0.02	0.85 $\pm$ 0.04	0.24 $\pm$ 0.03
Isoprenaline (10 $\mu$ g/kg)	-	-	-	30 $\pm$ 5	-	-	140 $\pm$ 20	-	0.78 $\pm$ 0.02	-

Each value represents the mean  $\pm$  SEM; n = 5; \*p<0.05, significantly different from the control; -, no value (isoprenaline induced series of small waves confusing with P and T, only QRS remained distinguishable).



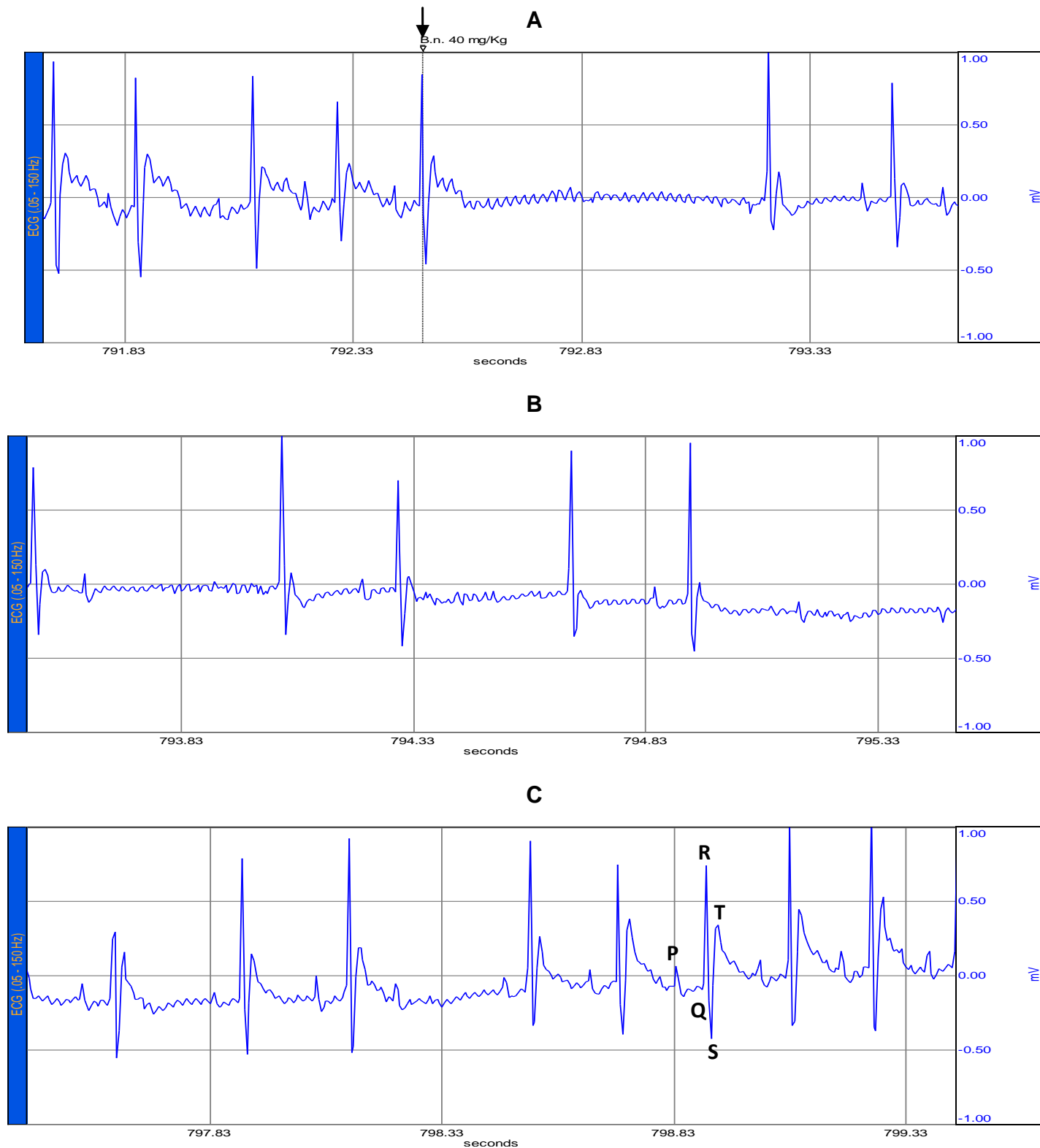
**Figure 3.** Effects of *B. nitens* (B.n., 40 mg/kg) methylene chloride/methanol extract on ECG of normotensive rat. (A) Changes in ECG immediately after *B. nitens* methylene chloride/methanol extract (B.n., 40 mg/kg) injection into rat's vein; (B) normal state recovering about 4 s after extract injection to rat; P, QRS and T are various waves of normal ECG.

In addition, as shown in table 2, neither atropine (1 mg/kg) nor propranolol (100 µg/kg) caused any significant ( $p > 0.05$ ) modification of the action of *B. nitens* extract on ECG parameters. Similarly, in reserpine (5 mg/kg/day) pre-treated rats, the elongation of R-R interval due to *B. nitens* (40 mg/kg) was not significantly inhibited. The 20.59% (from  $170 \pm 10$  to  $205 \pm 10$  min) R-R interval elongation was reduced non significantly ( $p > 0.05$ ) by 71.43% (maintaining the time at  $180 \pm 10$  min). A non significant ( $p > 0.05$ ) 50% inhibition of the extract induced P wave magnitude decrease was also observed in

the same animals.

#### Effects of the methylene chloride/methanol extract of *B. nitens* on hypertensive rats ECG parameters

In DSHR, injection of *B. nitens* extract (40 mg/kg) induced significant changes on T wave time, R-R interval and P wave magnitude (Figure 4; Table 3). On T wave, *B. nitens* extract caused a change of duration from  $90 \pm 20$  to  $50 \pm 10$  min, representing a 44.44% ( $p < 0.05$ )



**Figure 4.** Effects of *B. nitens* (B.n., 40 mg/kg) methylene chloride/methanol extract on DOCA-salt hypertensive rat ECG. (A) Changes in ECG immediately after *B. nitens* methylene chloride/methanol extract (B.n., 40 mg/kg) injection into rat's vein; (B) Fall down of the isoelectrical line 2 s after extract injection; (C) Recovery of steady state 6 s after extract injection to rat; P, QRS and T are the various waves of normal ECG.

**Table 2.** Effects of *B. nitens* methylene chloride/methanol extract, reference drugs and their antagonists on ECG of normotensive rats.

ECG parameter	Time (min)							Magnitude (mV)		
	P wave	P-R interval	P-R segment	QRS complex	Q-T interval	T wave	R-R interval	P wave	Complex QRS (R)	T wave
<i>B. nitens</i> (40mg/kg)	30 ± 4	50 ± 9	20 ± 5	30 ± 5	105 ± 20	75 ± 10	205 ± 10	0.15 ± 0.01	0.84 ± 0.01	0.16 ± 0.01
Res + <i>B. nitens</i>	35 ± 7	50 ± 8	20 ± 7	30 ± 7	110 ± 20	80 ± 10	180 ± 10	0.17 ± 0.01	0.87 ± 0.01	0.17 ± 0.01
Atro + <i>B. nitens</i>	30 ± 6	50 ± 8	20 ± 4	30 ± 7	115 ± 20	85 ± 10	210 ± 10	0.16 ± 0.01	0.85 ± 0.01	0.17 ± 0.02
Propra + <i>B. nitens</i>	30 ± 5	50 ± 8	20 ± 7	30 ± 5	110 ± 20	80 ± 10	205 ± 10	0.15 ± 0.01	0.85 ± 0.01	0.16 ± 0.01
Actylcholine (10 µg/kg)	30 ± 7	50 ± 6	20 ± 6	30 ± 6	80 ± 20	50 ± 20	240 ± 20	0.19 ± 0.02	0.85 ± 0.04	0.24 ± 0.03
Atro + Acetylcholine	30 ± 5	50 ± 4	20 ± 3	30 ± 8	110 ± 10	80 ± 10	180 ± 10*	0.19 ± 0.03	0.85 ± 0.04	0.19 ± 0.01
Isoprenaline (10 µg/kg)	--	--	--	30 ± 5	--	--	140 ± 20	--	0.78 ± 0.02	--
Propra + Isoprenaline	30 ± 7	50 ± 4	20 ± 5	30 ± 4	110 ± 10	80 ± 10	180 ± 10*	0.19 ± 0.03	0.85 ± 0.02	0.19 ± 0.01

Each value represents the mean ± SEM; n = 5; \*p<0.05, significantly different from the control (acetylcholine; isoprenaline); *B. nitens* (40 mg/kg) was the control for Res + *B. nitens*, Atro + *B. nitens* and Propra + *B. nitens*; - means no value; Atro = atropine (1 mg/kg); Propra = propranolol (100 µg/kg); Res = reserpine (5 mg/kg/day).

**Table 3.** Comparative effects of *B. nitens* methylene chloride/methanol extract (40 mg/kg) on electrocardiogram of normotensive and hypertensive rats.

Animal	Parameter	Time (min)						Magnitude (mV)			
		P wave	P-R Interval	P-R Segment	QRS Complex	Q-T Interval	T wave	R-R Interval	P wave	QRS (R) Complex	T wave
NTR	Control	30 ± 8	50 ± 8	20 ± 8	30 ± 6	110 ± 10	80 ± 10	170 ± 10	0.19 ± 0.03	0.85 ± 0.04	0.19 ± 0.01
	<i>B. nitens</i> (40 mg/kg)	30 ± 4	50 ± 9	20 ± 5	30 ± 5	105 ± 20	75 ± 10	205 ± 10*	0.15 ± 0.01*	0.84 ± 0.01	0.16 ± 0.01*
DSHR	Control	30 ± 10	50 ± 10	20 ± 10	30 ± 10	120 ± 10	90 ± 20	160 ± 10	0.20 ± 0.02	0.85 ± 0.04	0.29 ± 0.02
	<i>B. nitens</i> (40 mg/kg)	30 ± 10	50 ± 10	20 ± 10	30 ± 10	80 ± 20	50 ± 10*	320 ± 30**	0.16 ± 0.02*	0.85 ± 0.04	0.24 ± 0.03

Each value represents the mean ± SEM; n = 5; \*p<0.05 and \*\*p<0.01, significantly different from the control.

decrease. On R-R interval, the variation was from 160 ± 10 to 320 ± 30 min, representing a 100% (p<0.01) increase. More also, on P wave magnitude, the variation induced by *B. nitens* extract was from 0.20 ± 0.02 to 0.16 ± 0.02 mV, representing a 20% (p<0.05) decrease.

## DISCUSSION

This study investigated the effects of *B. nitens* CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH leaf extract on the ECG, as a possible mechanism contributing to its hypotensive activity in rat. Our results indicate that

*B. nitens* extract induced dose-dependent biphasic decrease of rat arterial blood pressure. These results are similar to those obtained by Dimo et al. (1999, 2003), who demonstrated that the methanol extract and the neutral extract of *Bidens pilosa*, respectively, had such biphasic

hypotensive effects in anaesthetized rats. Abdul-Ghani and Amin (1997) also found similar results while evaluating the effects of the aqueous extract of *Commiphora opobalsamum* on blood pressure and heart rate in rats. The earlier phase was brief and deep while the later phase was sustained. The latter was due to vasorelaxation induced by *B. nitens* extract, as we previously showed in normotensive rats (NTR) (Bopda et al., 2007; Dimo et al., 2007).

Prompt drops in both heart rate and blood pressure have been demonstrated for several medicinal plant extracts after their administration to rat (Corallo et al., 1997; Dimo et al., 2003; Kitjaroennirut et al., 2005). We earlier reported a possible contribution of cardiodepressive activity of *B. nitens* extract to the rapid phase of its hypotensive effects. In NTR and DOCA-salt hypertensive rats (DSHR), *B. nitens* (40 mg/kg) induced a 20.59 and 100% increase of R-R interval time, respectively. This corresponded to a decrease of heart rate, and confirms our previous results obtained by direct heart rate records (Bopda et al., 2007). In NTR, the extract caused a decrease of P wave and T wave magnitude; P wave and T wave are atria depolarization by nodal tissue and ventricle repolarization, respectively. Our results demonstrate that *B. nitens* extract inhibits atria depolarization and ventricle repolarization. We previously demonstrated on rat aorta smooth muscle that vasorelaxation induced by *B. nitens* extract relied on its calcium channels blocking action, which prevented the influx of extracellular calcium (Dimo et al., 2007). It is assumed nowadays that during the action potential of almost all cardiac muscle cells, depolarization is due to sodium influx, and directly followed by calcium influx in cardiac muscle cells. *B. nitens* also elicited a hypotensive action and a P wave magnitude decrease in DSHR. Salt given to those DSHR was NaCl and DOCA is known as a mineralocorticoid, thus has ability to retain sodium ions in rat internal medium. The higher the sodium concentration in the internal medium, the easier the muscle cells depolarization could be. Results demonstrate that *B. nitens* extract might have inhibited sodium influx and/or calcium influx, and then reduced ability of atria muscle cells to depolarize or to elicit a proper action potential.

Furthermore, the decrease of T wave magnitude reinforced this issue, since during a normal heart contraction, ventricles repolarization is a prerequisite for a new atria depolarization. The decrease of T wave time observed in DSHR was due to the global miniaturization of the ECG duration in the presence of extract, which contributes to lowering the capacity of ventricle to totally repolarise, and hence make it difficult for the next depolarization by nodal tissue. Thus, it obviously appears that the extract exerted a depressive action on electrical activity of cardiac muscle, and then on its contraction. The relaxation of

cardiac muscle might happen, as in aorta muscle, via a calcium channels blockade.

Atropine and propranolol are muscarinic and  $\beta$ -adrenoceptor blockers, respectively (Dimo et al., 2003; Bopda et al., 2007; Kouakou et al., 2008). The cardiodepressive activity of *B. nitens* extract was not affected by those blockers. Neither did the extract act via muscarinic receptors nor via  $\beta$ -adrenoceptors. Similar results were reported by Eno and Owo (1999) in their study on the cardiovascular effects of an *Elaeopphorbia drupifera* roots extract. The negative chronotropic effect of the extract, as well as its decreasing effect on the magnitude of P wave, were partially (but not significantly) inhibited by reserpine (5 mg/kg/day). These results might express a possible interaction between the extract and reserpine, but not on cardiac  $\beta$ -adrenergic receptors. Thus, the main possible mechanism of action of *B. nitens* extract should be a direct calcium channels inhibition, as we suggested previously (Bopda et al., 2007). This might be associated with a blockade of sodium channels. While investigating the effects of some *Calotropis procera* extracts on the activity of diverse rabbit muscles, Moustafa et al. (2010) also suggested that the ethanol extract might act directly on the myocardium, inducing a negative chronotropic effect.

In our previous works, we explained that the calcium blockade caused by *B. nitens* extract on vessels is due to some alkaloids (Bopda et al., 2007; Dimo et al., 2007) and also suggest that the same molecules might be responsible for the direct calcium channels inhibition on the cardiac pump. The cardiodepressive activity of *B. nitens* extract is rather partial vis-a-vis of its hypotensive effect, since only the higher dose (40 mg/kg) was significantly efficient.

More also, our results relating to acute toxicity indicate that *B. nitens* extract was not toxic when administered to mice by oral route. Similar results were reported by Akah et al. (2009, 2010) while evaluating the acute toxicity of the aqueous extract and that of the methanol fractions of the leaves of *B. nitens* in mice. All the changes on heart activity occurred within less than 10 s following extract administration. The recovery observed in all the animals, as well as the non-lethality up to 8000 mg/kg of extract, gave proof that *B. nitens* causes no acute toxic effect in mice or rats. Those observations justified the fact that the early hypotensive phase of *B. nitens* extract was linked to a non-toxic depression on ECG parameters.

In conclusion, the hypotensive effects induced by the  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$  leaf extract of *B. nitens* rely on its cardiodepressive activity. The extract at a higher dose (40 mg/kg) caused, in NTR and DSHR, a negative chronotropic effect and a decrease of the magnitude of P wave. The extract might act by blocking calcium channels and possibly sodium channels.



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