

## Hypotensive action of an aqueous extract of *Pimenta dioica* (Myrtaceae) in rats

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Received 8-3-1999. Corrected 6-IX-1999. Accepted 9-IX-1999.

**Abstract:** The intra-venous (i.v.) hypotensive action of the final aqueous fraction of *Pimenta dioica* was studied in Spontaneously Hypertensive Rats (SHR). The rats were anaesthetized (sodium pentobarbital 50 mg/kg), the trachea, right carotid artery and jugular vein were cannulated for adequate ventilation, direct blood pressure measurement and intra-venous administration of extracts, solutions and drugs. The arterial line was connected to a pressure transducer (Viggo-Spectramed model P23 XL) and a polygraph (Grass model 7H) and monitored continuously during the first five minutes after plant extract administration and then at 5 and 15 minute intervals for one hour. Responses were taken as the maximum pressure changes observed during this period. Increasing doses of the final aqueous fraction were given i.v. to groups of six SHR each. It produced a dose dependent decrease in blood pressure and the ED<sub>50</sub> was 45 mg/kg. To discard that the hypotensive effect of the extracts was due to its ionic composition, a solution containing KCl, NaCl, CaCl<sub>2</sub> and MgCl<sub>2</sub> equivalent to the ion contents present in a dose of 50 mg/kg of total aqueous extract was injected to Sprague-Dawley rats (SDN) using the same method as described above. It did not produce significant changes in blood pressure. Pharmacological antagonistic studies were done injecting either autonomic ganglion,  $\alpha$  adrenoceptor,  $\beta$  adrenoceptor and cholinergic receptor blockers prior to extract administration in SHR rats. Atropine, propranolol and phentolamine did not affect the hypotensive effect of the final aqueous fraction. With hexamethonium (autonomic ganglion blocker) the hypotensive response was diminished in a significant way ( $p < 0.05$ ). The hypotensive action of the final aqueous extract was not mediated through cholinergic,  $\alpha$  or  $\beta$  adrenergic receptors. The extract may possess vasorelaxing activity which could not be evident after autonomic ganglion blockade due to extreme vasodilation present prior to extract administration. Future studies should address the question of a possible direct vasodilating effect of the extracts.

**Key words:** *Pimenta dioica*, traditional medicine, herbal medicine, antihypertensive effect, arterial hypertension.

*Pimenta dioica* (L.) Merrill (family Myrtaceae) is a tree native of the West Indies and Central America (Rogers 1963). The dried unripe berries are used mainly as spice and condiment (Grieve 1971). They are also used for flatulent indigestion, as a febrifuge and are considered to have tonic and antihelmintic properties (Germosén-Robineau 1995). The

leaves of this tree known popularly as "jamaica" have been used in Costa Rican folk medicine as an antihypertensive (Vargas 1990). It has been demonstrated that the administration of different extracts of *P. dioica* to conscious normotensive and hypertensive rats causes a depression of the central nervous system, which is dose dependent. Analgesic

and hypothermic effects have also been documented (Suárez *et al.* 1996-1997).

Phytochemical studies show the presence of essential oils and tannins in both the berries and the leaves. Alkaloids are absent (Dominguez *et al.* 1962).

The hypotensive activity of ethanolic and aqueous extracts of *Pimenta dioica* and several fractions of the aqueous extract has been observed in anaesthetized normotensive rats (Suárez *et al.* 1997). The intravenous (i.v.) administration of the aqueous extract of *Pimenta dioica* to these rats produced a dose related significant fall in mean arterial blood pressure (MAP) and the ED<sub>50</sub> was 54 mg/kg. The aqueous extract showed a greater hypotensive effect than the ethanolic extract. Of all the fractions of the aqueous extract tested, the final aqueous one seems to be the most effective in lowering arterial blood pressure (Suárez *et al.* 1997).

It is the purpose of this research to further study the hypotensive activity of the total aqueous extract of *Pimenta dioica* and its final aqueous fraction to acquire some knowledge on the mechanisms involved in this cardiovascular activity.

## MATERIALS AND METHODS

**Plant Material and Preparation of Extracts:** The leaves of *P. dioica* (L.) Merrill were collected in the province of Heredia, Costa Rica, during November of 1993 and January of 1994. A voucher specimen was deposited in the Herbarium of the University of Costa Rica (USJ 508060).

The fresh leaves (1 kg) were extracted with ten liters of distilled water at 70° C during 20 minutes. The extract was filtrated and concentrated to 2400 ml under vacuum at 40°C. Half of this concentrate was lyophilized to obtain 64.26 g of powder (13 % yield) which was called total aqueous extract. To obtain the final aqueous fraction, the other half was successively extracted with hexane, ethyl acetate and *n*-butanol obtaining 0.36 g (0.072 % yield), 2.90 g (0.58 % yield) and 14.75 g (2.95 % yield) respectively. The

solvents were then evaporated and the material lyophilized obtaining 28.55 g (5.71 % yield) of what we called the final aqueous fraction.

The total aqueous extract and final aqueous fraction were dissolved in 0.9% saline solution before intravenous administration to animals.

**a) Anaesthetized Rats:** Male spontaneously hypertensive rats (SHR) weighing between 225-275 g were anaesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg). The trachea was cannulated with a polyethylene tube to secure adequate ventilation. The right carotid artery was cannulated with heparinized polyethylene tubing PE-50 which was connected to a pressure transducer (Viggo-Spectramed model P23 XL) and a polygraph (Grass model 7H) for blood pressure recording. The right jugular vein was also cannulated for intravenous injection. An EKG (Lead II) was registered to calculate heart rate.

After a 15-min equilibration period, increasing doses (10, 30, 44, 62, 100 mg/kg) of the final aqueous fraction were given to groups of six rats each. Parameters were recorded continuously during the first five minutes after plant extract administration and then at 5 minute, and 15 minute intervals for one hour. Responses were taken as the maximum pressure changes observed during this period. A dose-response curve was made, the ED<sub>50</sub> was calculated and then used in pharmacological antagonistic studies.

**b) Ionic composition of the total aqueous extract:** The K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> and Mg<sup>2+</sup> contents of the total aqueous extract were estimated by atomic absorption spectrometry. A solution containing KCl, NaCl, CaCl<sub>2</sub> and MgCl<sub>2</sub> equivalent to the ion contents present in a dose of 50 mg total aqueous extract/kg was injected to six SDN anaesthetized rats to verify the effect of these ions on their blood pressure.

**c) Pharmacological antagonist studies:** To study the possible antihypertensive mechanism of the extracts of *P.dioica*, four groups of six SHR each were prepared for experimentation as outlined above but, autonomic

ganglion (group 1),  $\alpha$ -adrenoceptor (group 2),  $\beta$ -adrenoceptor (group 3) and cholinergic receptor (group 4) blockades were produced by using a specific blocker (group 1: hexamethonium 20 mg/kg, group 2: phentolamine 2 mg/kg, group 3: propranolol 0.4 mg/kg and group 4: atropine 1 mg/kg). The effectiveness of the receptor blockade was tested by injecting an agonist in an effective dose, then the specific antagonist was given and when the blood pressure was stabilized, the same dose of the agonist was given again. If the agonist's action was blocked, evidenced by a change in mean arterial pressure smaller than 5 %, a test dose of the final aqueous fraction (45 mg/kg) was then given immediately. Methoxamine (50  $\mu$ g/kg), isoproterenol (0.5  $\mu$ g/kg) and acetylcholine (1  $\mu$ g/kg) served as the  $\alpha$ ,  $\beta$  and cholinergic agonists respectively. The four groups were compared to a group of six SHR that only received the final aqueous fraction ( $ED_{50}$ : 45 mg/kg). This was the control group. A last group received an equivalent volume of 0.9 % saline solution.

**Drugs:** The following agents were used: Acetylcholine chloride (Merck), Phentolamine methanesulfonate (CIBA Geigy), methoxamine hydrochloride (Burroughs Wellcome Co.), isoproterenol hydrochloride (Sigma), propranolol hydrochloride (Sigma), atropine (Sigma) and hexamethonium chloride (K & K Laboratories, Inc.).

**Statistics:** All values were expressed as mean  $\pm$  SEM. Blood pressure values obtained with the different doses of the extract and the group that received the ionic solution, were compared by analysis of variance (ANOVA). To verify the effect of the blockers used in the pharmacological antagonist studies a paired t-test was used and for the comparison of the response of the different blocked groups to the final aqueous fraction a General Linear Model Procedure was applied using SAS. The level of significance was defined at  $p < 0.05$ .

## RESULTS

**Dose-Response Curve:** When increasing doses of the final aqueous fraction were given

intravenously to anaesthetized spontaneous hypertensive rats a dose related significant decrease in mean arterial blood pressure was observed. The  $ED_{50}$  was 45 mg/kg. (Fig. 1).

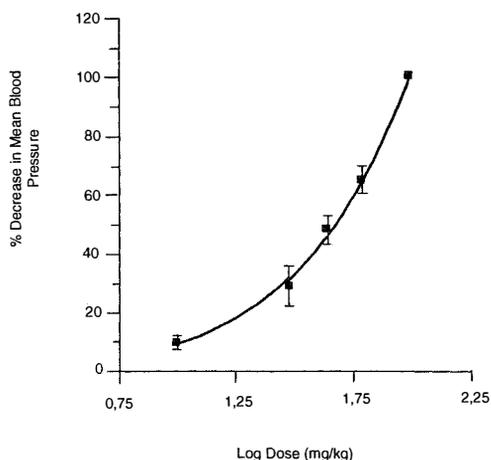


Fig. 1. Dose related effects of *P. dioica* final aqueous fraction on the mean blood pressure of Spontaneously Hypertensive Rats. Each point corresponds to the mean  $\pm$  SEM of six rats.

There was no significant change in heart rate except with the 100 mg/kg dose, which caused bradycardia and death. No abnormalities in the EKG were observed.

**Effect of the ionic composition of the total aqueous extract:** The  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$  and  $Mg^{2+}$  contents were 30, 4.3, 1.1 and 3.3 mg/g of the total aqueous extract respectively. The intravenous injection of a solution containing KCl, NaCl,  $CaCl_2$  and  $MgCl_2$  equivalent to the ion contents present in the total aqueous extract; did not produce significant changes in mean arterial blood pressure of SDN anaesthetized rats and the effect was never different from the one observed with a 0.9 % saline solution.

**Pharmacological antagonist studies:** Atropine, propranolol and phentolamine that blocked the effects of acetylcholine, isoproterenol and methoxamine respectively, did not affect the hypotensive effect of the extract. When hexamethonium was given, the hypotensive response to the extract was diminished in a significant way ( $p < 0.05$ ) (Fig 2).

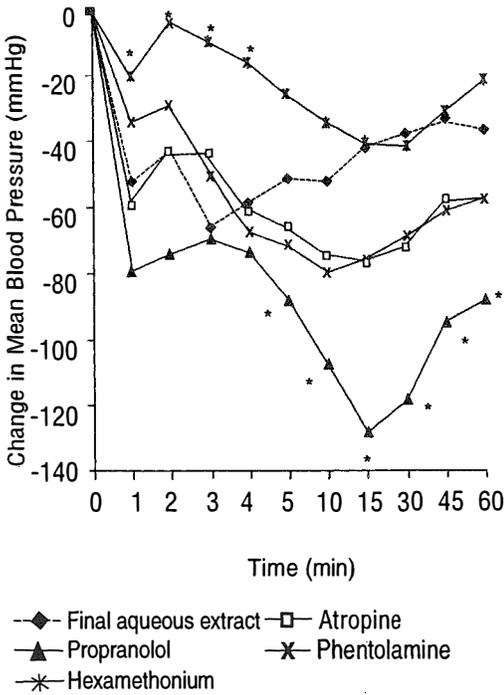


Fig.2. Effect of  $\alpha$  and  $\beta$  adrenergic, cholinergic and autonomic ganglion blockade on mean blood pressure (mmHg) of SHR during 60 minutes after the  $ED_{50}$  of the final aqueous fraction of *P. dioica* was given. The control group only received the  $DE_{50}$  of the fraction. Each point represents the mean of six rats. \*  $p < 0.05$ .

To simplify the understanding of the results, the changes in mean blood pressure and heart rate were analyzed in three different periods of time after extract administration. Immediate changes observed during the first minute, the period between the first and fifth minute and a later 55 minute time lapse that ends 60 minutes after extract administration.

The first 30 seconds after extract administration were carefully examined and every five seconds the mean blood pressure decreased an average of 5, 9, 10, 5 mmHg for the phentolamine, propranolol, atropine and hexamethonium blocked groups respectively. There was no significant difference between the groups or between them and the control group. Heart rate also decreased but again there was no significant difference between the

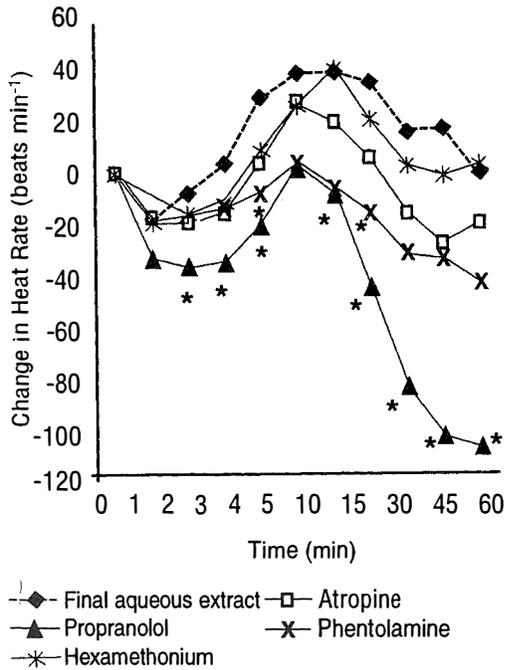


Fig.3. Effect of  $\alpha$  and  $\beta$  adrenergic, cholinergic and autonomic ganglion blockade on heart rate (beats/min) of SHR during 60 minutes after the  $DE_{50}$  of the final aqueous fraction of *P. dioica* was given. The control group only received the  $DE_{50}$  of the fraction. Each point represents the mean of six rats. \*  $p < 0.05$ .

groups or between the groups and the control or the 0.9% saline solution group.

In all the groups, the greatest change in mean blood pressure was observed at the end of the first minute after extract administration (Fig.2) and at this time, all the groups presented a decrease in heart rate which was not significantly different between them (Fig. 3).

In the control group the greatest hypotension was seen during the third minute after extract administration. After this, the mean blood pressure showed a recuperating tendency until the end of the sixty minute observation period.

Between the first and the fifth minute after extract administration, only the hexamethonium group failed to show the hypotensive effect of the extract in a significant way ( $p < 0.05$ ) (Fig.2).

Heart rate, after the initial first minute bradycardia, did not decrease any more and the general trend in all groups was to increase to maximum values around the fifth to tenth minute after extract administration. The propranolol group always had significantly lower heart rates (Fig.3).

Between five minutes and the end of the observation period, in all the groups the mean blood pressure showed a recuperating tendency associated with reflex tachycardia. The propranolol group always had significantly ( $p < 0.05$ ) lower mean blood pressures between five and 60 minutes after extract administration, and it was not before 15 minutes that a clear recuperating tendency of the blood pressure was observed. The other groups started the recuperation process earlier and reached higher pressures at the end of the 60 minutes in a similar way to the control group (Fig.2). At the end of the sixty minutes, none of the groups had recovered mean blood pressure to control levels.

Heart rate recovered completely at the end of 60 minutes in the control group and in the hexamethonium group. The propranolol group had difficulties in recuperating heart rate and at the end of the observation period had a significant bradycardia ( $p < 0.05$ ) (Fig.3).

## DISCUSSION

The final aqueous fraction of *P. dioica* has a dose related hypotensive effect on SHR. The  $ED_{50}$  is smaller (45 mg/kg) than the  $ED_{50}$  for the total aqueous extract on SDN rats (54 mg/kg) (Suárez *et al.* 1997). This could be due to the fact that the antihypertensive compound is more concentrated in the fraction than in the total aqueous extract.

The hypotensive action of the final aqueous extract was not mediated through cholinergic,  $\alpha$  or  $\beta$  adrenergic receptors. Atropine, phentolamine and propranolol, which almost completely blocked the effects of acetylcholine, methoxamine and isoproterenol respectively, did not affect the depressor effect of the extract.

When hexamethonium was given, the hypotensive response to the extract was diminished in a significant way ( $p < 0.05$ ) (Fig.2), this means that the autonomic ganglion blockade diminished the hypotensive effect of the extract.

The initial bradycardia observed during the first minute after extract administration is probably due to non-specific factors because it was seen in all groups including the control and 0.9 % saline solution groups. A direct unspecific effect on the heart could be the cause. The intravenous injection in the jugular vein of a solution at room temperature could well explain this bradycardia (Smith *et al.* 1984). The bradycardia does not seem responsible for the hypotensive effect of the extract because the animals that received 0.9 % saline solution presented it without hypotension. Peripheral vasodilation may be the cause of the extract effect. The extract may present a vasorelaxing activity, which could not be evident after autonomic ganglion blockade due to the extreme vasodilation presented prior to extract administration. Probably, for the same reason, the phentolamine treated group, presented less hypotension although the difference with the control group was not significant. A direct effect of the extract on the vascular smooth muscle could be involved. It has been described (Calixto 1986) that tannic acid may cause hypotension in rats due to nonspecific depressor effects in vascular smooth muscle and *P. dioica* is a tannin-rich plant (Dominguez *et al.* 1962).

The reduction in blood pressure may also be due to non-specific factors other than the active principles present in the extract. Data show that the depressor effect of *P. dioica* is independent of the amount of  $K^+$ ,  $Ca^{2+}$ ,  $Na^+$  or  $Mg^{2+}$  ions in the total aqueous extract.

The extract does not seem to affect the capacity of the heart to increase heart rate in order to compensate after the initial hypotension. Only very large doses of the extract cause persisting bradycardia and death.

An inotropic effect of the extract cannot be ruled out.

A peripheral effect of the extract is favored by the fact that hypotension persists at important levels between five and sixty minutes after extract administration even though there is a reflex tachycardia.

Future studies must be made with extracts which have been submitted to tannin removing techniques and the possible direct vasodilating effect of the extracts should be tested using isolated organ methods.

#### ACKNOWLEDGMENTS

We thank Jorge Gómez-Laurito (University of Costa Rica) for the classification of the plant material and Norman R. Farnsworth (University of Illinois at Chicago) for his help with the NAPRALERT database. This work was supported by the grant from the Universidad de Costa Rica No 422-93-243.

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