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Full Length Research Paper

Cardiovascular activity of total protein extract of *Morinda morindoïdes* (baker) Miln-Redhon on rabbit blood pressure and isolated rat heart

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Total protein extract of the leaves of *Morinda morindoïdes* were investigated for rabbit blood pressure and mechanical activity of isolated rat heart. Total proteins of *M. morindoïdes* (PT-Mm) were extracted according the saturation related to Dawson method. The blood pressure was analysed using the mercury manometer of Ludwig. The mechanical activity of the heart was realized with the Langendorff method. PT-Mm produced a reversible hypotension at doses ranging from 5.55 to 27.77 mg/kgBw, and irreversible hypotension to 55.55 mg/kg resulting in the death of the animal. PT-Mm showed a significant decrease (31.77 ± 2.72%) in average of the arterial blood pressure in a dose-related manner. Also, PT-Mm (10^{-8} to 10^{-1} mg/ml) decreased both the force and rate of myocardial contraction, respectively (28.58 ± 2.47 and 33.35% ± 2.35) in a concentration dependent manner. The hypotensive effect of PT-Mm was inhibited by atropine sulfate. The hypertensive effect of adrenaline was strongly reduced by PT-MN extract (77.44 ± 2.23%). In conclusion, total proteins extract of *M. morindoïdes* has blood pressure lowering effect which would result from the combined actions of myocardial depression and activation of muscarinic cholinergic receptors mediated by vascular smooth muscle relaxation.

Key words: Cardiovascular activity, *Morinda morindoïdes*, Total proteins, Blood pressure, Heart rate.

INTRODUCTION

Cardiovascular diseases are the main causes of death among developed countries. More than 50% of cardiovascular deaths are related to coronary artery diseases (CAD) (Ali et al., 2004; Vafadar and Soltani, 2005; Jiang et al., 2005; American Heart Association, 2009).

Many plant species are used in folk medicine to manage hypertension due their hypotensive properties. Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health cure (Austin, 1991). Medicinal plants have over the years constituted indispensable tools for the research and the development of new drugs medications (Krogsgaard-Larsen et al., 1984). Furthermore, WHO accepted to classify plants medications in the general health program until year 2000 (Deputy, 2002).

Morinda morindoïdes (baker) Miln-Redh (Rubiaceae) which is well known in the traditional medicine practice of tropical countries has interested us for this study. Many works have been published on the pharmacological properties of *M. morindoïdes*. In Democratic Republic of Congo, M. *morindoïdes* was used for the treatment of several diseases, including: Malaria, diabetes, microbial infections and dermatis (Cimanga et al., 2006). The population of Ivory Coast having diarrhea was treated with *M. morindoïdes* (Meite et al., 2009). Previous studies reported that the extracts of *M. morindoides* had various pharmacological activities such as antimicrobial (Bagre et al., 2011), spasmolytic (Cimanga et al., 2010), antimalarial

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(Cimanga et al., 1997, 2003, 2008), cardioinhibitory (N'Guessan et al., 2002), antiamoebic (Cimanga et al., 2006) and immunologic (Mankele et al., 2006).

Total extract of *M. morindoïdes* in oral way, is used traditionally in Ivory Coast for diarrhea treatment; this total extract contains several molecular components. Being given the typical effects that might have bioactive molecules on cardiovascular activity such as polyphenol compounds, peptides (Omer et al., 2011; Yang et al., 2011), it was important to assess pharmacological activities about each group of molecular components of this plant, to prevent the risk of toxicity. In this effect, we focused on proteins that play an important function in our body including: immunity system, transport, cellular structure, catalysis enzymatic reaction etc.

The present study investigates on cardiovascular properties of total extract proteins of *M. morindoïdes* and eventually examines the possible mechanism by which these proteins express their effects.

MATERIALS AND METHODS

Plant material

The leaves of *M. morindoïdes* (Rubiaceae) were collected from Daloa (central west region of Ivory Coast) in June 2009. The plant was identified and authenticated by Pr Ake Assi, of the Department of Botany; University of Cocody. A voucher specimen (n^o 17710) of the plant was deposited in the herbarium of the National Floristic Center of the University of Cocody-Abidjan.

Animal condition

Rabbits (both sexes 12 to 16 weeks old weighing 1.5 to 2 kg) and rats (both sexes, 6 to 8 weeks old, weighing 0.2 to 0.3 kg) bred at the department of biosciences (University of Cocody-Abidjan, Ivory Coast), were used for the experiments. All animals were kept at constant humidity (60%) and temperature (25°C) in a 12 h light/dark cycle with free access to food and water.

Animals were cared for and treated according to the principles of the use of laboratory animals. Approval for the studies was given by the ethical committee of the University of Cocody-Abidjan. The equipment, handling and sacrificing of the animals were in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals (Mitjans et al., 2008). Before the experiment, rabbits were deprived from food for 24 h but given free access to water.

Drugs and chemicals

The drugs used were: Urethane, heparin adrenaline acetylcholine and atropine sulfate. All chemicals were purchased from Sigma Chemicals Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steineheim, Germany), and Merck (Darmstadt, Germany).

Preparation of the leaves for PT-Mm extraction

M. morindoïdes leaves were cleaned of extraneous matter, air-dried at room temperature for 7 days and ground into a fine powder. The powder was mixed with distilled water (80 g of powder in 2 L of distilled water) for 24 h with constant stirring at fresh. The extract was filtered twice through cotton wool, then through Whatman filter paper (No.1).

The filtrates saturated with ammonium sulfate to 80 or 90% saturation according to the method of Dawson et al. (1986) for the precipitation of proteins. After homogenization, the solution is kept for 24 h and subsequently centrifuged 4000 trs/min. A fraction that was obtained shows two phases: An upper phase (supernatant) and a lower phase (the sediment) which was our protein extract. The sediment collected was dialyzed against distilled water (Wilson and Walker, 1994) with a synthetic membrane of dialysis. The presence of proteins in the dialyzed pellet was revealed by the Lowry test (Lowry et al., 1951). Dried protein solution was freeze-dried for better storage stability (Osterlund and Jonson, 1997) and the resulting powder had constituted our total proteins extract of *M. morindoïdes* leaves (PT-mm).

Experimental protocol

Recording of the arterial blood pressure

On the day of the experiment, rabbits were anesthetized with ethylurethane (40 at a dose of 1 g/kg per body weight) administered intraperitoneally. Through an incision in the neck region, the trachea was exposed and cannulated for clear airway and spontaneous respiration. Thereafter, the saphenous vein and carotid artery were isolated and cannulated for drug administration and blood pressure recording, respectively. After cannulation, the rabbits were injected with 1000 IU of heparin to prevent blood clotting. After the period of equilibration, the rabbits were injected with 1 ml of saline solution (NaCl: 0.009 mg/mL) or with the same volume of PT-Mm. Arterial blood pressure was allowed to return to the resting level between injections. The changes in blood pressure before and after injection were analyzed. The possible interaction between the hypotensive action of *M. morindoïdes* extract, and systems (cholinergic and adrenergic) was studied. To do that, we have injected PT-Mm immediately after treatment with different doses of the muscarinic receptor blocker atropine ($5.55\ 10^{-7}$ to $5.55\ 10^{-4}$ mg/kgBw), or immediately before treatment with appropriate agonist adrenaline about $5\ 10^{-3}$ mg/kgBw.

Recording of the mechanical heart activity

For the study of the mechanical heart activity, the experimental was carried out in accordance with the Langendorff procedure. After anesthezing with 20% of ethylurethane, a rat was sacrificed, dissected and the aortawas exposed and cannulated with a syringe containing heparinized Tyrode solution to extract the rat heart. The heart was quickly isolated after median thoracotomy and attached to the infusion device. A small spring clip was attached to the apex of the ventricle and connected to a cardiac transducer which transmits the cardiac movements to a recording cylinder covered with smoked paper. The perfusion fluid was Tyrode solution (in mM: NaCl (130.5); KCl (5.63); $CaCl_2$ (2.16); $MgCl_2$ (0.24); NaH_2PO4 (1.18); $NaHCO_3$ (11.90) and glucose (11.10)), which was continuously bubbled with oxygen. The fluid was applied at constant flow rate from a reservoir maintained at 37°C by water circulated through thermostated water bath. The extract was administered in the perfusion in graded concentrations of 10^{-8}, 10^{-6}, 10^{-4}, 10^{-2} and 10^{-1} mg/ml.

Statistical analysis

Data were analyzed by one-way ANOVA followed by Dennett's

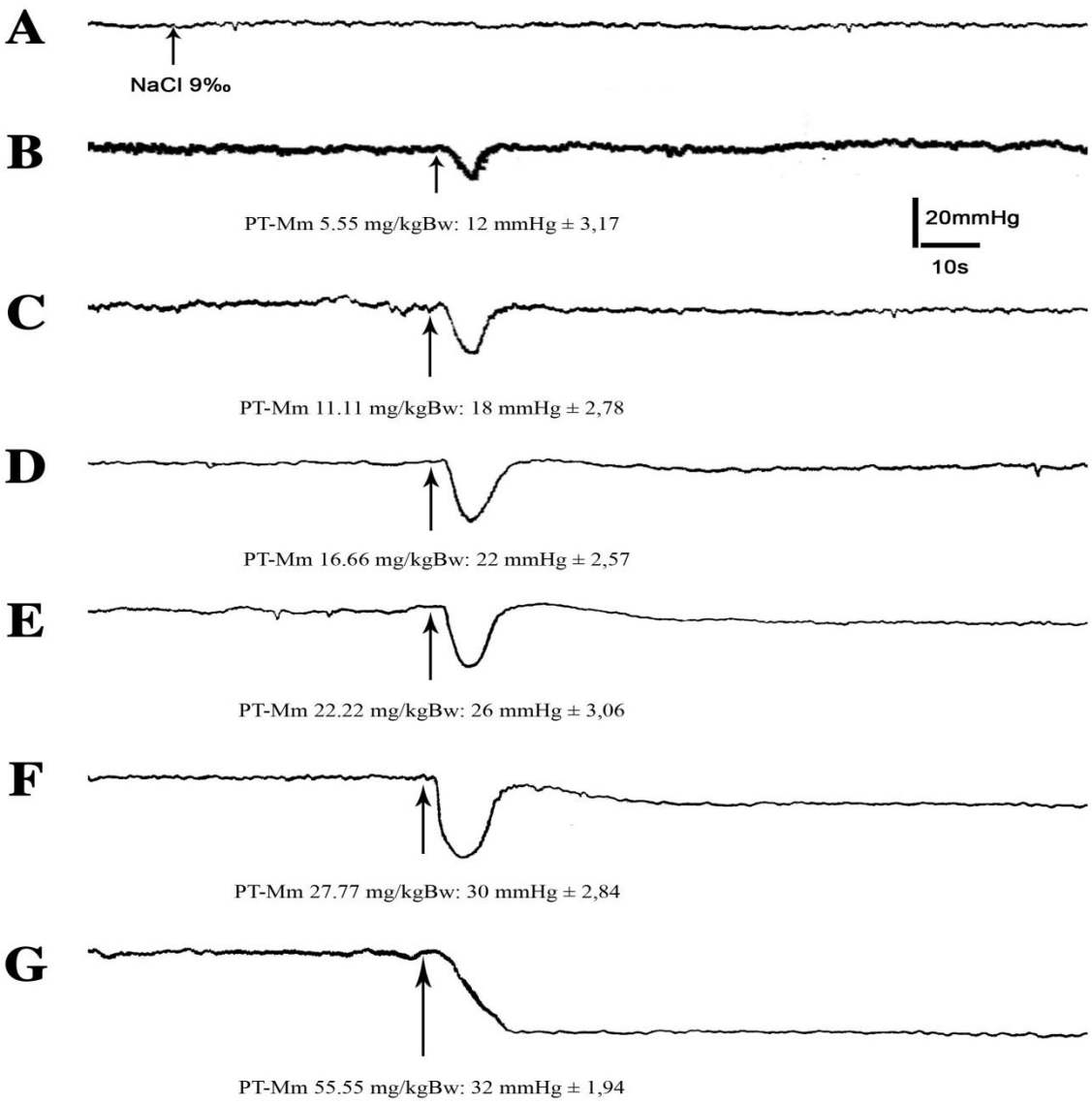

Figure 1. Dose response effect of PT-Mm on mean arterial blood pressure of rabbit. A: Effect of NaCl 9‰ (control recording); B to G: effect of PT-Mm.

t-test using instat (Graph Pad software, USA). A p value of < 0.05 was considered statistically significant.

RESULTS

Effect of PT-Mm extract on the rabbit arterial blood pressure

In normal anesthetized rabbit, the intravenous injection of total proteins extracts of *M. morindoïdes* (mg/kgBw) produced almost immediately a significant reduction on average of the arterial blood pressure ($p < 0.05$). The hypotensive effect of PT-Mm was dose-dependent and reversible for the doses ranging from 5.55 to 27.77 mg/kgBw, as shown in Figure 1. Higher doses of the extract about 55.55 mg/kgBw produced a more significant decrease and irreversible effect on arterial blood pressure. The EC_{50} of about 16.48 mg/kgBw was determined from the curve (Figure 2).

Antagonistic effect of atropine, adrenaline and PT-Mm

The recording in Figure 3 represents the interaction between PT-Mm (mg/kgBw) and increasing concentration of atropine. The reduction of blood pressure was significantly inhibited by all doses tested of atropine (5.55 10^{-7} to 5.55 10^{-4} mg/kgBw). The interaction of ascending

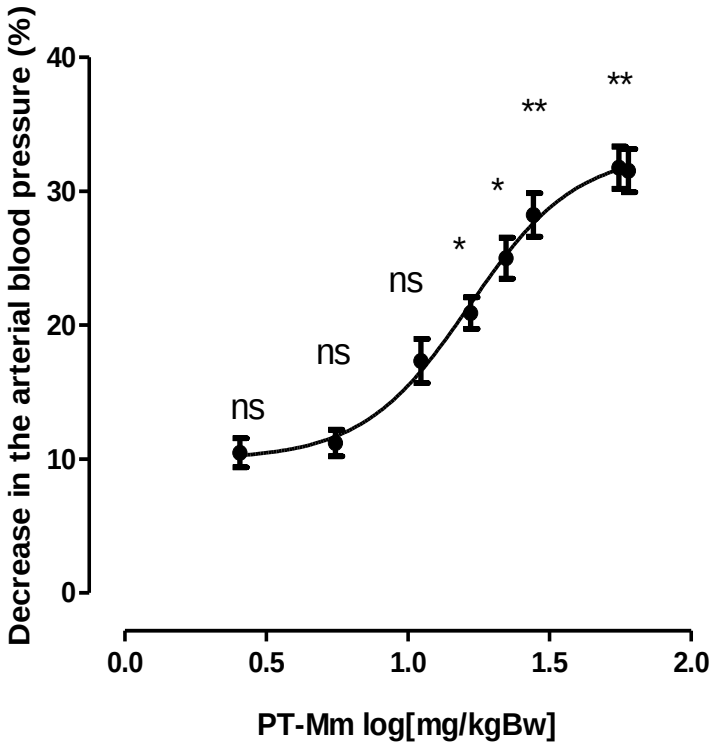

Figure 2. Dose response curve showing the decrease of arterial blood pressure according the different doses of PT-Mm. Each point is the mean ± SE (n = 3). *, $p < 0.05$; **,$p < 0.01$ compared to control.

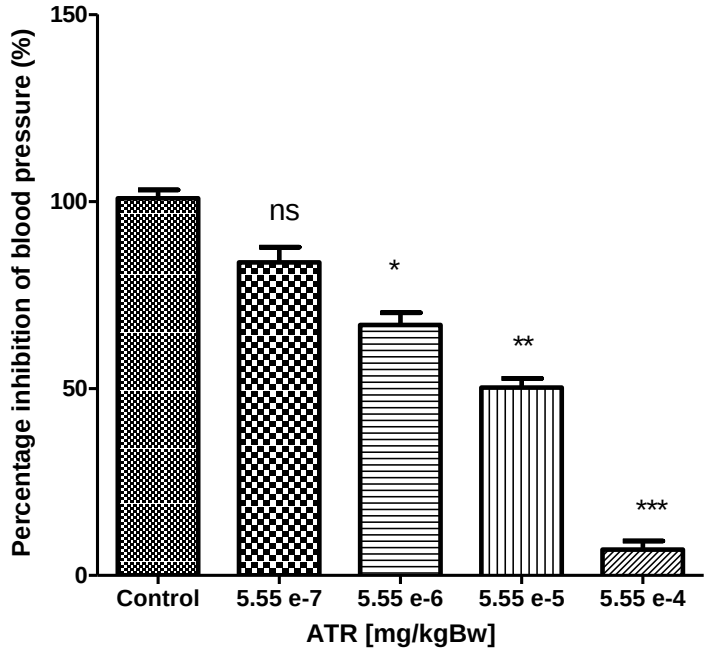

Figure 3. The inhibitor effect of Atropine on the hypotensive effect of PT-Mm. Values are expressed as mean ± S.E.M (n = 3); *, $p < 0.05$; **, $p < 0.01$; ***$p < 0.001$ compared to control. X e-a = X.10^{-}.

doses of the PT-Mm extract immediately after treatment with adrenaline produced significant effect on arterial blood pressure. PT-Mm decreased the hypertensive effect of adrenaline on rabbit blood pressure as shown in Figure 4.

Effect of PT-Mm extract on the heart mechanical activity

PT-Mm extract showed a depressant effect on the heart rate and the amplitude of the heart contractions. The effect was significant ($p < 0.05$) and dose dependent with an acute value of about 10^{-1} mg/ml shown in Figure 5 from an initial level of 36 beats/min to 24 beats/min.

DISCUSSION

PT-Mm extract of *M. morindoïdes* lowered rabbit carotid arterial blood pressure. On rabbit blood pressure, PT-Mm induced a reversible hypotension at doses ranging from 0.55 to 27.77 mg/kgBw, and irreversible hypotension to 55.55 mg/kgBw resulting in the death of the animal. This observation agrees with the works realized by N'Guessan et al. (1995, 2002, 2004), who found that the total aqueous extracts and fraction F5 of *M. morindoïdes* induces a dose-dependent hypotension. On the other hand, the effect of PT-Mm on the mechanical activity of the isolated rat heart, revealed cardioinhibitor action characterized by negative inotropic and chronotropic dose-dependent effects. Both the rate and force of myocardial contractility were depressed in a concentration dependent manner, with a greater effect on the rate than the force of contraction. The decrease in carotid blood pressure obtained with the PT-Mm, suggests that these proteins would reduce the activity of cardiac contractile and/or vascular resistance. Our results are in concordance with the findings of Salahdeen et al. (2004) and Fabien and Eric (2007) respectively on the extracts of *Tridax procumbens* and *Phyllanthusamarus*.

Furthermore, the antagonism of the hypotensive effect of the extract by atropine pretreatment would seem to suggest the involvement of cholinergic mechanism in the action of the extract. The cholinomimetic compounds contained in PT-Mm would exert their hypotensive effect through cardiac or vascular muscarinic cholinergic receptors. It is important to note that the hypotensive action of PT-Mm on arterial blood pressure was similar to that of acetylcholine (ACh). In effect, the binding of ACh on muscarinic receptors induces cardioinhibition manifested by both a reduction in myocardial contractile force and heart rate (Hanf et al., 1993). Meanwhile, the stimulation of vascular muscarinic receptors by ACh induces vasodilatation by the relaxation of vascular smooth muscle (Furchgott, 1981). On the other hand, the depressor activity of PT-Mm on artery blood pressure

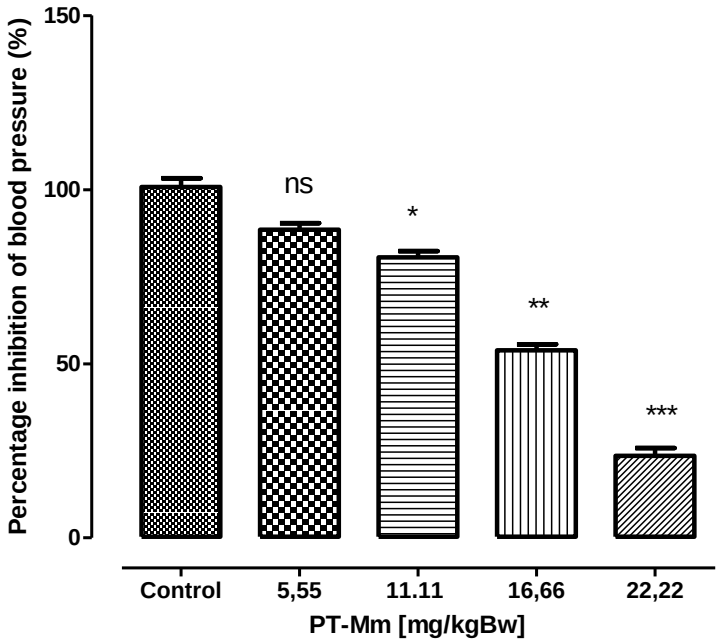

Figure 4. The antihypertensive effect of PT-Mm on high blood pressure induced by Adrenaline. Values are expressed as mean ± S.E.M (n = 3). *, p < 0.05; **p < 0.01; ***P < 0.001 compared to control.

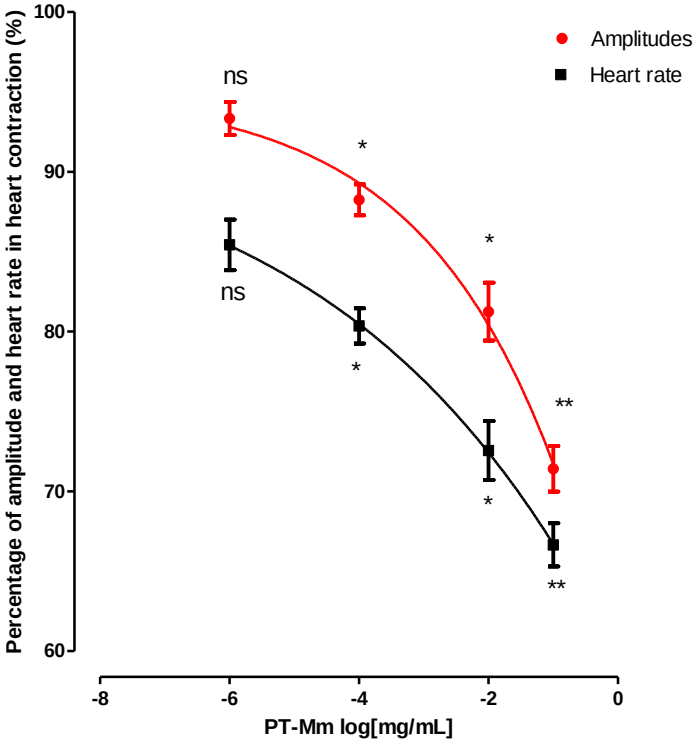

Figure 5. The percentage of amplitude and heart rate in the mechanical activity of the heart. Each point is the mean ± SE (n = 3). *, P < 0.05; **, P < 0.01 compared to control.

would be dependent on adrenergic receptors. In effect, our investigation to know if adrenaline receptors used by *M. morindoïdes* proteins showed that adrenaline effect was diminished when we have injected total proteins of *M. morindoïdes*. We could suggest that total proteins of *M. morindoïdes* would compete with adrenaline receptors to exert their antihypertensive action. This indication suggests that the protein extract would have same antihypertensive activity as *Berberis vulgaris* (Fatehi-Hassanabab et al., 2005). Also it is important to know that ADR produces vasoconstriction and high heart rate which increase blood pressure. The hypertensive effect of ADR was explained by the binding of ADR on beta-adrenoreceptors. Thus the inhibition of the effect of ADR on blood pressure by PT-Mm would suggest the presence of beta blocker compounds in PT-Mm extract of *M. morindoïdes*. PT-Mm could be explored to find new treatments against high blood pressure and cardiomyopathy such as other bioactive molecules including polyphenols and peptides compounds (Omer et al., 2011; Yang et al., 2011).

In Conclusion, PT-Mm extract of *M. morindoïdes* exerts hypotensive and antihypertensive activities that would result from the combined action of vascular smooth muscle relaxation, and the depression of myocardium about the force and the rate of contraction. PT-Mm is a constituent of total extract of *M. morindoïdes*, which have the same property than total extract. Thus to prevent the risk of toxicity that might result from the combination of all molecules of total extract of *M. morindoïdes*, it would be interesting to further study these proteins. This primer study realized is an investigation; we will in our future studies characterize these proteins and identify the presence of cholinomimetic and their beta blocker compounds.

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