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Hypotensive mechanism of the extracts and artemetin isolated from *Achillea millefolium* L. (Asteraceae) in rats

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ABSTRACT

Traditional uses of *Achillea millefolium* L. (Asteraceae) include the treatment of cardiovascular diseases. In the present study, we used anesthetized rats to assess the hypotensive effect of a hydroethanolic extract (HEAM), and its dichloromethane (DCM), ethyl acetate (EA), butanolic (BT), and dichloromethane-2 (DCM-2) fractions, besides the flavonoid artemetin, isolated from *A. millefolium*. The oral administration of HEAM (100–300 mg/kg), DCM (20 mg/kg), DCM-2 (10–30 mg/kg), but not EA (10 mg/kg) and BT (50 mg/kg) fractions significantly reduced the mean arterial pressure (MAP) of normotensive rats. The phytochemical analysis by NMR ^{1}H of DCM and DCM-2 fractions revealed high amounts of artemetin, that was isolated and administered by either oral (1.5 mg/kg) or intravenous (0.15–1.5 mg/kg) routes in rats. This flavonoid was able to dose-dependently reduce the MAP, up to 11.47 ± 1.5 mm Hg (1.5 mg/kg, i.v.). To investigate if artemetin-induced hypotension was related to angiotensin-converting enzyme inhibition, we evaluated the influence of this flavonoid on the vascular effects of both angiotensin I and bradykinin. Intravenous injection of artemetin (0.75 mg/kg) significantly reduced the hypertensive response to angiotensin I while increased the average length of bradykinin-induced hypotension. Artemetin (1.5 mg/kg, p.o.) was also able to reduce plasma (about 37%) and vascular (up to 63%) ACE activity *in vitro*, compared to control group. On the other hand, artemetin did not change angiotensin II-induced hypertension. Our study is the first showing the hypotensive effects induced by the extract and fractions obtained from *A. millefollium*. In addition, our results disclosed that this effect may be, at least in part, associated with high levels of artemetin and its ability to decrease angiotensin II generation *in vivo*, by ACE inhibition.

1. Introduction

Achillea millefolium L. (Asteraceae) is a perennial herb that has been extensively used in folk medicine around the world (Chandler et al., 1982; Wichtl and Bisset, 1989). In Brazil, it is popularly known as “mil-folhas”, “novalgina”, “erva-de-carpinteiro”, “aquileia”. This plant is traditionally indicated against gastrointestinal disorders (Benedek and Kopp, 2007; Nemeth and Bernath, 2008), liver and gall-bladder conditions (Haggag et al., 1975; Miraldi et al., 2001), menstrual irregularities (Newall et al., 1996), fever (Blumenthal et al., 2000), wound healing (Aljancic et al., 1999), and cardiovascular diseases (Font Quer, 1988; Martínez, 1996). Several traditional uses have been investigated in experimental studies, including anti-inflammatory (Benedek et al., 2007), analgesic (Pires et al., 2009), anti-ulcer (Cavalcanti et al., 2006), and hepatoprotective (Yaeesh et al., 2006). Although preparations obtained from this plant have been widely used as antihypertensive and diuretic by human subjects, there are no scientific data supporting none of these applications.

Phytochemical screenings revealed that chemical constituents of *A. millefolium* L. presents several secondary metabolites, including essential oil (cineol, borneol, pinenes, camphor, menthol, eugenol, azulen, and chamazulene; Lorenzi and Matos, 2002; Panizza, 1997), sesquiterpenes (paulitin, isopaulitin, psilostachyin C, desacetylmatricarin, and sistenin; Csupor-Loffler et al., 2009; Glasl et al., 2002), the alkaloid achilleine (Miller and Chow,

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1954), steroids (β-sitosterol, stigmasterol, cholesterol, campesterol; Chandler et al., 1982), triterpenes (α-amyrin, β-amyrin, taraxasterol, pseudotaraxasterol; Chandler et al., 1982), and flavonoids (such as centaureidin, casticin, apigenin, luteolin, rutin, quercetin, acacetin, isorhamnetin, and artemetin; Csupor-Loffler et al., 2009; Guédon et al., 1993; Teske and Trentini, 1997). A number of these substances had beneficial effects evidenced in several pathological conditions.

Taking into account the lack of studies regarding the cardiovascular effects of *A. millefolium*, we have investigated if the hydroethanolic extract obtained from its aerial parts, as well as semi-purified fractions, would be able to induce any changes in blood pressure of anesthetized rats. Using a bio-guided approach we have characterized that the most active fraction was rich in artemetin (5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone). This flavonoid has been scarcely studied regarding its biological effects, but antioxidant (Dugas et al., 2000) and, anti-inflammatory (Sertie et al., 1990) properties, as well as its ability to inhibit the cell cycle (Li et al., 2005), and the enzyme lipoxygenase (Choudhary et al., 2009) have been previously established. Nevertheless, there are no studies investigating the effects of artemetin in the cardiovascular system. As our results will show, artemetin seems responsible, at least in part, for the anti-hypertensive action attributed to *A. millefolium*, an effect mediated by angiotensin-converting enzyme inhibition.

2. Material and methods

2.1. Drugs and reagents

Angiotensin I, angiotensin II, bradykinin, captopril and losartan were all purchased from Sigma (St. Louis, MO, USA). Bradykinin, and angiotensins I and II (stock solution) were dissolved in HCl (0.1 N). All other drugs were freshly prepared in saline solution (0.9%).

2.2. Animals

Male Wistar rats (220–280 g), supplied by Universidade Federal do Paraná (UFPR, Curitiba, PR, Brazil), were housed in a temperature- and light-controlled room (22 ± 2 °C; 12 h light/dark cycle), and acclimatized in our laboratory for a period of at least 12 h before any experiment, with free access to water and food. All procedures were approved by the Institutional Ethics Committee of UFPR (authorization number 240).

2.3. Plant material

A. millefolium used in our experiments was collected in July 2007 from the botanical garden of Universidade Paranaense (UNIPAR, Umuarama, PR, Brazil), Umuarama (Brazil) at 430 m altitude above sea level (S23°47′55–W53°18′48). The plant was identified by Dr. Mariza Barion Romagnolo (Department of Botany, UNIPAR). Voucher specimens were deposited at the Herbarium of UNIPAR under number 2230.

2.4. Preparation of the hydroethanolic extract (HEAM)

Dried and powdered aerial parts of *A. millefolium* were extracted with 90% ethanol at room temperature. The solution was concentrated in a vacuum rotation evaporator, filtered and lyophilized to give the crude hydroalcoholic extract (HEAM-yielding 17.39%).

2.5. Isolation of the essential oil and bio-guided isolation of artemetin

The essential oil (yielding 0.16%) was obtained from the leaves of *A. millefolium* by hydrodistillation (3 h) with a clevenger type apparatus.

The HEAM was suspended in EtOH–H_2O (1:1) and then extracted with dichloromethane (DCM), ethyl acetate (EA), and 1-butanol (BT), successively. The solvent evaporation yielded the fractions DCM (20%), EA (10%), and BT (50%). Since the hypotensive activity described in this study was concentrated in the fraction DCM, an aliquote (4.4 g) of this fraction was submitted to vacuum column chromatography in silica gel, eluted with solvents of crescent polarity (petroleum ether, CH_2Cl_2, CH_2Cl_2–MeOH (3:1) and pure methanol), to give four fractions named F1 (0.06 g), F2 (1.22 g), F3 (2.68 g), and F4 (0.74 g). Our data revealed that the activity remained in fraction F2 (DCM-2 sub-fraction). This fraction was subjected to silica gel chromatography column eluted with increasing amounts of ethyl acetate in hexane, giving 20 fractions, which were subjected to TLC analysis. The fraction 17 (yielded 60 mg) was very rich in artemetin (almost pure). It was purified by repeated silica gel preparative TLC eluted with hexane–CH_2Cl_2–MeOH (2:1:0.1) to give pure artemetin (13.8 mg), a flavonoid previously reported in *A. millefolium* (Csupor-Loffler et al., 2009). This compound was identified by ^1H and ^{13}C NMR (200 MHz, $CDCl_3$) under comparison with previously reported data (Barberá et al., 1986). In our *in vivo* experiments, artemetin was dissolved in a sodium bicarbonate solution (0.5%), and the final volume completed with saline (0.9%).

2.6. Direct blood pressure measurement in anesthetized rats

Male *Wistar* rats were anesthetized with ketamine (100 mg/kg) and xylazine (20 mg/kg), given by the intramuscular route, and supplemented at 45–60 min intervals. A polyethylene catheter was inserted into the right femoral vein for drug administration. Immediately after venous access a bolus injection of heparin (30 IU) was administered. The animals were allowed to breath spontaneously through a tracheostomy. The left carotid artery was cannulated and connected to a pressure transducer coupled to a MacLab® recording system, and its application program (Chart, v 4.1), all from ADI Instruments (Castle Hill, Australia), allowing the recording of the mean arterial blood pressure (MAP). For stabilization of the blood pressure after the surgical process, an interval of 15 min was held before the injection of any drug. At the end of experiments, animals were killed with an overdose of thiopental (over 40 mg/kg, i.v.).

2.7. Administration of A. millefolium extracts, fractions, and artemetin under MAP measurement

In these experiments a single dose of HEAM (100 mg/kg, p.o.), or vehicle (CT), were administered to the animals at 1.5, 3, 6 and 12 h before the animals were prepared for MAP measurement, as previously described. In another set of experiments, different animals received a single oral dose of HEAM (30 and 300 mg/kg), butanolic (BT-50 mg/kg), ethyl acetate (EA-10 mg/kg), dichloromethane fractions (DCM-20 mg/kg), DCM-2 subfraction (3, 10 and 30 mg/kg), or artemetin (1.5 mg/kg). Control animals (CT) received vehicle only. All these groups were subjected for MAP measurements at 3 h after treatments.

The pressoric effects of artemetin after intravenous administration were also evaluated. For this, anesthetized rats received artemetin, at doses of 0.15, 0.35, 0.75 and 1.5 mg/kg (i.v.). An interval of at least 10 min was allowed between each injection. Control

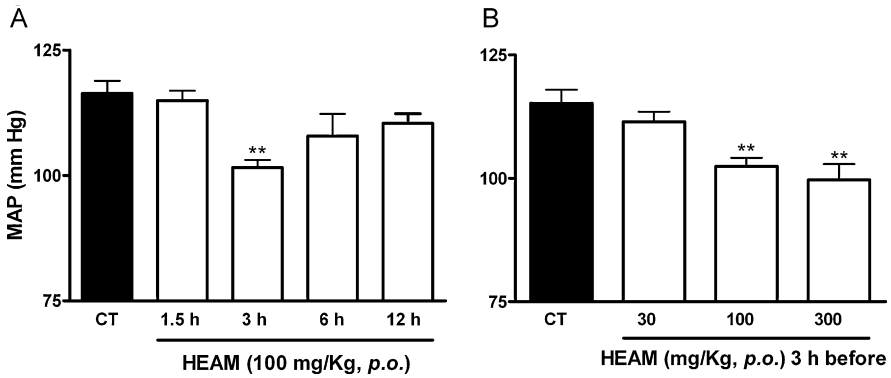

Fig. 1. Hypotensive effect induced by *A. millefolium* preparations in rats. (**A**) The HEAM was administered by oral route at different times before MAP measurement. (**B**) Change in MAP (mm Hg) of anesthetized rats, orally treated 3 h prior, with HEAM. The "CT" refers to the control group, treated with vehicle only. The results show the mean ± S.E.M. Statistical analyses were performed by means of one-way analysis of variance (ANOVA) followed by *t*-test subjected to Bonferroni's correction. $^{**}p < 0.01$ when compared to control group.

animals received vehicle only. The dosages adopted in these experiments were calculated based on the yield from the extract.

2.8. Influence of artemetin in the dose-response effects of angiotensin I, angiotensin II, and bradykinin

All drugs used in this experiment were dissolved in saline and were given in a total 200 μl intravenous bolus. After baseline assessment and stabilization of MAP for 30 min, the rats received intravenous injections of angiotensin I (Ang I), or angiotensin II (Ang II), both at doses of 0.1, 1 and 3 nmol/kg, or bradykinin (BK; 10 nmol/kg). These doses were chosen accordingly to previous studies (Lima-Landman et al., 2007; Montenegro et al., 2009) and our own experience. After the measurement of the regular effects of these drugs, different groups of animals received a single intravenous injection of artemetin (0.75 mg/kg), captopril (10 nmol/kg), losartan (1 mg/kg), or saline (CT). Five minutes after these treatments the injections of Angio I, Angio II, and BK were repeated, and compared with those effects obtained before.

2.9. Angiotensin-converting enzyme (ACE) assay

The animals were orally treated, 3 h prior, with artemetin (1.5 mg/kg), captopril (20 mg/kg) or control (vehicle). Blood was collected into glass tubes, and serum was separated by centrifugation (3000 rpm) by 15 min. Briefly, serum (10 μl) was incubated with 490 μl of assay solution containing 5 mM Hip-His-Leu in 0.4 M sodium borate buffer, pH 8.3, and 0.9 M NaCl for 15 min at 37 °C. The reaction was stopped by addition of 1.2 ml of NaOH 0.34 N. The product, His-Leu, was measured fluorometrically (365 nm excitation and 495 emission, Aminco Model J4-7461 fluoromonitor, American Instrument Co., Silver Springs, MD) after the addition of 100 μl of o-phthaldialdehyde (20 mg/ml) in methanol, which was followed 10 min later by the addition of 200 μl of HCl 3 N and centrifugation at 800 × *g* for 5 min at room temperature. To correct the intrinsic fluorescence of the plasma, time-zero blank samples were prepared by adding plasma after NaOH treatment. All measurements were made in triplicate.

2.10. In vitro assessment of vascular response to angiotensin I

For this experiment, we used the descending thoracic aortas of these animals treated for blood collection (as described above). Isolated aorta rings were prepared according to the standard procedures previously described (Da Silva-Santos et al., 2002). Tension was recorded via isometric force transducers (Letica Scientific Instruments, Barcelone, Spain) coupled to a MacLab® recording system (MacLab/8) and an application program (Chart versus 3.3), both from ADI Instruments (Castle Hill, Australia), working on an Apple Computer®. After one hour the preparations were exposed to angiotensin I (1 nM–10 μM)-induced vasoconstriction. The values of vasoconstriction (in grams) of control group were compared with the results obtained in the animals that received artemetin or captopril treatment.

2.11. Statistical analysis

Results are expressed as the mean values ± S.E.M. (standard error of mean) of four to eight animals. The statistical evaluation was carried out using Student's *t*-test or one-way analysis of variance (ANOVA) followed by Bonferroni's *t*-test. In all analysis, a *p* value < 0.05 was considered as statistically significant. Graphs were drawn and statistical analyses were carried out using GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Reduction of blood pressure by the hydroethanolic extract from A. millefolium (HEAM)

In control animals the basal MAP recorded was 116.4 ± 2.5 mm Hg. The MAP was significantly smaller in those animals that received HEAM (100 mg/kg, *p.o.*) 3 h (but not 1.5, 6 or 12 h) before the experiments, about 13 ± 1 mm Hg (Fig. 1A). The highest dose of HEAM tested in our experiments (300 mg/kg, *p.o.*) decreased the mean arterial pressure by 14 ± 3 mm Hg (Fig. 1B), while the oral (30, 100 or 300 mg/kg) or endovenous (3, 10 or 30 mg/kg) administration of essential oil obtained from *A. millefolium* did not alter the MAP after 3 h from each administration (results not shown).

3.2. Effects of fractions obtained from HEAM and artemetin isolated from A. millefolium in the mean arterial pressure of anesthetized rats

The oral administration of DCM fraction (20 mg/kg), 3 h before blood pressure measurement, significantly reduced the MAP of anesthetized rats, when compared to the control group (treated with vehicle only) in 11 ± 1 mm Hg. On the other hand, neither the butanolic (BT, 50 mg/kg, *p.o.*), nor the ethyl acetate (EA, 10 mg/kg, *p.o.*) fractions caused similar blood pressure reduction (Fig. 2A). The F2, DCM-2 sub-fraction (that was obtained from DCM fraction), reduced the mean arterial pressure by 10 ± 1 mm Hg

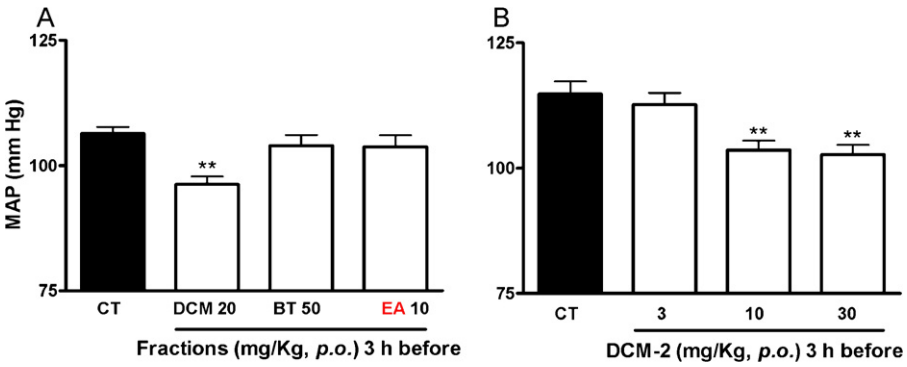

Fig. 2. Hypotensive effect induced by DCM fraction and DCM-2 sub-fraction in rats. (**A**) Change in MAP (mm Hg) of anesthetized rats, orally treated 3 h prior, with DCM, BT and EA fractions. (**B**) Hypotensive effect induced by DCM-2 sub-fraction (administered orally 3 h before the experiment). The "CT" refers to the control group, treated with vehicle only. The results show the mean ± S.E.M. Statistical analyses were performed by means of one-way analysis of variance (ANOVA) followed by *t* test subjected to Bonferroni's correction. $^{**}p < 0.01$ when compared to control group.

when orally administered, 3 h before blood pressure measurements (Fig. 2B). In addition, artemetin isolated from F2 (DCM-2 sub-fraction) presented a similar ability to reduce the mean arterial pressure when orally administered 3 h before the experiments, and caused a dose-dependent hypotensive effect when intravenously administered (0.15, 0.35, 0.75 and 1.5 mg/kg; *i.v.*), as illustrated in Fig. 3C, with reductions about 8 ± 3 mm Hg per oral route (Fig. 3B) and 5.6 ± 0.91 to 11.47 ± 1.5 mm Hg by intravenous administration (Fig. 3B). Artemetin (Fig. 3A) was found as the main component of DCM and DCM-2 by analysis of NMR ^{1}H spectra of these fractions.

3.3. Effects of artemetin on vascular responses to angiotensin I, angiotensin II and bradykinin

In order to determine if the hypotensive effect of artemetin could be related to any action on the renin-angiotensin system, we carried out an *in vivo* protocol to evaluate the activity of angiotensin-converting enzyme (ACE). Injection of artemetin (0.75 mg/kg) reduced the hypertensive effects of Ang I by ~40–50%, when compared to the control group (Fig. 4A). Similarly, the average length of the effects of Ang I was decreased in artemetin-treated rats (Fig. 4B). The effects of artemetin on Ang I-induced hypertension were very similar to that observed in animals treated with captopril (10 nmol/kg) (Fig. 4A and B). In addition, administration of artemetin (as well as captopril) increased the average length of vasodilatation induced by bradykinin (Fig. 4D), without change the peak of vasodilatation induced by this drug (Fig. 4C). Differently than losartan, artemetin was unable to avoid the hypertensive effects of Ang II (Fig. 6).

3.4. Artemetin induces change in plasma and vascular ECA activity

We found significant changes in plasma ECA activity in rats pre-treated with artemetin and captopril (101.8 ± 12 his-

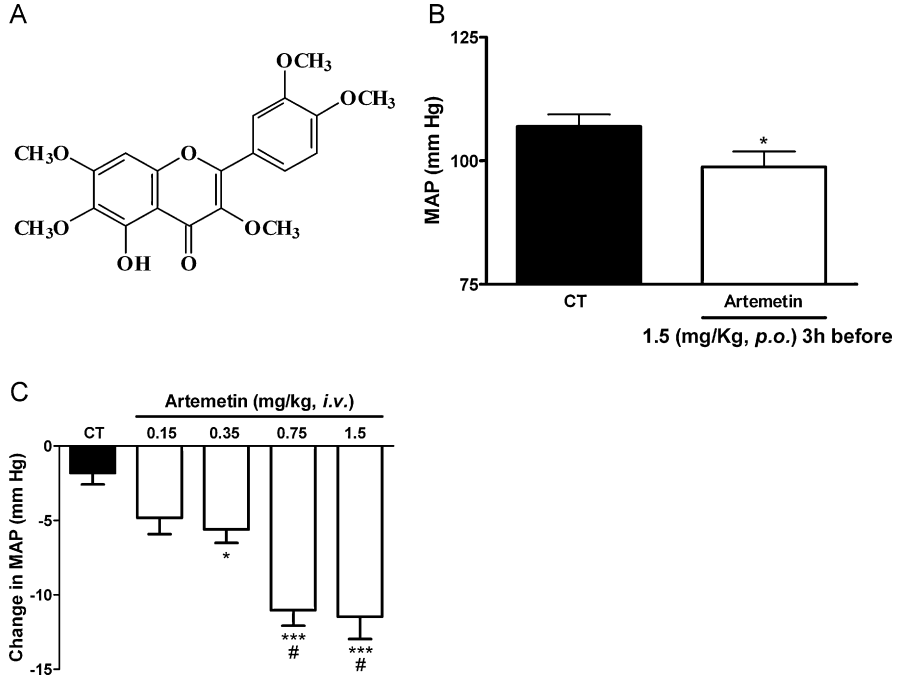

Fig. 3. Hypotensive effect induced by artemetin isolated from *A. millefolium* in rats. (**A**) Chemical structure of methoxylated flavonoid artemetin. (**B**) Change in MAP (mm Hg) in anesthetized rats pretreated (by oral route) with artemetin. (**C**) Dose-dependent hypotensive effect of artemetin intravenously administrated in rats. The "CT" refers to the control group, treated with vehicle only. The results show the mean ± S.E.M. Statistical analyses were performed by means of one-way analysis of variance (ANOVA) followed by *t* test subjected to Bonferroni's correction (panel C), or unpaired Student's *t*-test (panel B). $^{*}p < 0.05$, $^{***}p < 0.001$ when compared to control group. #$p < 0.05$ when compared to artemetin 0.35 mg/kg group.

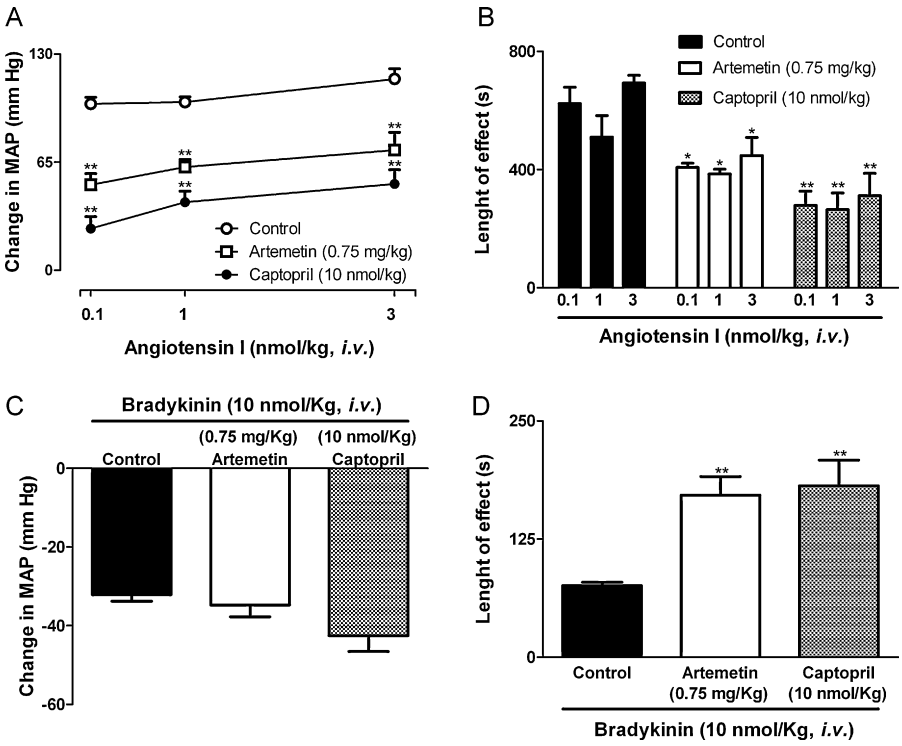

Fig. 4. Artemetin reduces the vasoconstriction induced by angiotensin I and increases vasodilatation by bradykinin. Change in MAP (**A**) and average length of the hypertensive effect (**B**) of angiotensin I in anesthetized rats, after intravenous administration of artemetin or captopril. Change in MAP (**C**) and prolongation of bradykinin effects by artemetin and captopril administration (**D**). Control groups received vehicle only. The results show the mean ± S.E.M. Statistical analyses were performed by means of one-way analysis of variance (ANOVA) followed by *t*-tests subjected to Bonferroni's correction. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared to control group.

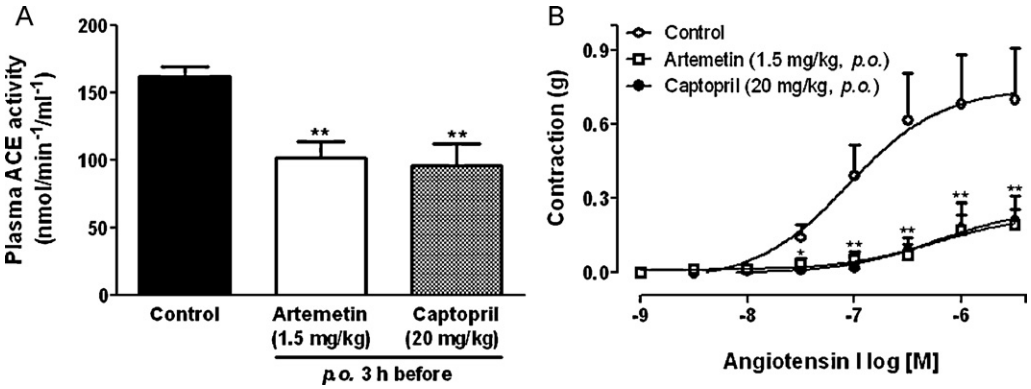

Fig. 5. Vascular angiotensin-converting enzyme (ACE) activity inhibition by artemetin. Rats were treated with vehicle (control), artemetin and captopril, per oral route (3 h before). (A) ACE activity was assessed using a fluorometric method with the use of Hyppuryl-His-Leu as substrate. (B) The isolated aorta rings were exposed to angiotensin I induced vasoconstriction. Statistical analyses were performed by means of one-way analysis of variance (ANOVA) followed by *t*-test subjected to Bonferroni's correction. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared to control group.

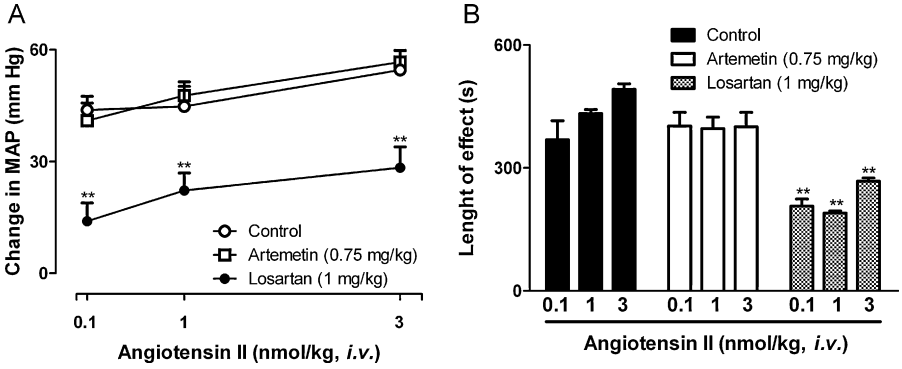

Fig. 6. Artemetin does not block angiotensin II-induced hypertension. Change in MAP (**A**) and average length of the hypertensive effect (**B**) of angiotensin II in anesthetized rats after intravenous administration of artemetin or losartan. Statistical analyses were performed by means of one-way analysis of variance (ANOVA) followed by *t* test subjected to Bonferroni's correction. $^{*}p < 0.05$, $^{**}p < 0.01$ when compared to control group.

leu nmol/min^{-1}/ml^{-1} and 96 ± 16 his-leu nmol/min^{-1}/ml^{-1}) compared with those found in plasma samples from rats in the control group (161.9 ± 7.8 his-leu nmol/min^{-1}/ml^{-1}) (Fig. 5A). In addition, artemetin caused a great reduction in vasoconstrictor response to Ang I in aorta rings, about 63–89% when compared to control group. Similar result was observed with captopril treatment (Fig. 5B).

4. Discussion

The results of the present study did show that the extract and semi-purified fractions (DCM and DCM-2) obtained from *A. millefolium* L. (Asteraceae), a plant popularly used against cardiovascular diseases in several countries, including Brazil, were able to reduce the blood pressure of rats, after oral administration. Furthermore, the hypotensive effect of the crude extract and fractions could be associated with high levels of artemetin, a methoxylated flavonoid previously identified in *A. millefolium* (Csupor-Loffler et al. 2009), and also found in other plants, such as *Artemisia arborescens* (Abu Zarga et al., 1995), and *Cordia curassavica* DC (Bayeux et al., 2002).

It has been described that several flavonoids and flavonoid-rich fractions present beneficial effects in the cardiovascular system, including vasodilatation (e.g. Ajay et al., 2003; Ajay et al., 2007; Dong et al., 2009; Lemos et al., 1999), antioxidant (e.g. Engler et al., 2004; Heim et al., 2002; Modak et al., 2005; Prasad et al., 2009; Wang et al., 2010) and antihypertensive activities (e.g. Cho et al., 2007; Magos et al., 2008). Nevertheless, the cardiovascular effects of artemetin have never been described. Thus, this is the first study showing that oral or intravenous administration of artemetin reduces, in a dose-dependent manner, the mean arterial pressure of rats, suggesting that this flavonoid is the main responsible for the cardiovascular effects popularly attributed to *A. millefolium*.

A single dose of artemetin, as low as 1.5 mg/kg, did reduce the MAP of rats in a range of ~10 mm Hg, even when orally administered 3 h before the measurement of blood pressure. It has been estimated that, in humans suffering from hypertension, a reduction of 5 mm Hg in MAP can decreases the mortality due to stroke and coronary events by 14 and 9%, respectively (Chobanian et al., 2003; Stamler, 1991; Whelton et al., 2002), reinforcing the importance of our findings (Fig. 5).

Preparations obtained from *A. millefolium* are continuously self-administered as a phytomedicine. Although neither *A. millefolium* nor artemetin have been chronically administered in our experiments. Further to our interest, a pioneering study showing toxicological experiments, where sub-acute oral administration of artemetin was evaluated in rats, indicated a very low toxicity of this flavonoid (Sertie et al., 1990).

The reduction in blood pressure disclosed in this study after a single administration of fractions obtained from *A. millefolium* suggests that this practice may contributes in the treatment of hypertension in humans. Importantly, we did not evaluate the detrimental effects of this practice, and this study was fully developed in rats. Thus, the safety and efficacy of *A. millefolium* in humans remain to be investigated.

Interestingly, administration of artemetin did reduce both the average length of effect and the peak of hypertension induced by angiotensin I in rats, in a way very similar to captopril, a well-known ACE inhibitor (Fig. 4). The vasoconstriction induced by angiotensin II is dependent of its ability to binding and activates AT1 subtype receptors (Ninahuaman et al., 2007). However, our results indicate that artemetin does not act as an antagonist of AT1 receptors, since differently than losartan (an AT1 receptor antagonist), artemetin did not reduce the effects of angiotensin II in blood pressure. In addition, the hypotension induced by bradykinin remained for an extended period of time in those animals treated with either artemetin or captopril. Taken together with the reduction of angiotensin I-induced vasoconstriction elicited by artemetin both *in vivo* and *in vitro*, as well as both orally and intravenously administered, these data suggest that the hypotensive effect of this flavonoid is, at least in part, due to ACE inhibition. Our data are in accordance to scientific literature, where several flavonoids have been reported to inhibit ACE activity (Actis-Goretta et al., 2003; Kameda et al., 1987; Lacaille et al., 2001; Oh et al., 2004; Wagner, 1993).

Hypertension is, in most cases, a chronic condition that almost always must be daily treated with anti-hypertensive drugs. Although the current clinical practice guidelines states lowering blood pressure as a priority for people suffering from hypertension (Lonn, 2004), the efficiency of the pharmacological treatment is reduced by absence of satisfactory responses, or non-adherence for anti-hypertensive drugs regimens, that may be related to adverse effects or even the financial cost of these therapies. At least to our known, this is the first study showing that preparations obtained from *A. millefolium*, and the flavonoid artemetin, are able to cause blood pressure reduction in rats. Artemetin is not the only active compound, but it showed activity and certainly contributes to the antihypertensive effect of crude extract.

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