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To link to this article: http://dx.doi.org/10.3109/13880209.2015.1107103

Published online: 05 Jan 2016.

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Beneficial effect of medicinal plants on the contractility of post-hypoxic isolated guinea pig atria – Potential implications for the treatment of ischemic–reperfusion injury

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ABSTRACT

Context Ischemic–reperfusion injury is accompanied by a decreased contractility of the myocardium. Positive-inotropic agents have proven useful for treating this condition but may exert serious side-effects.

Objective In this study, aqueous preparations from Abelmoschus esculentus L. Moench (Malvaceae), Annona muricata L. (Annonaceae), Bixa orellana L. (Bixaceae), Cecropia peltata L. (Moraceae), Erythrina fusca Lour. (Fabaceae), Psidium guajava L. (Myrtaceae) and Terminalia catappa L. (Combretaceae) were evaluated for their ability to improve the decreased contractility of isolated guinea pig atria after hypoxic stress.

Materials and methods Guinea pig atria isolated in Ringer-Locke buffer gassed with 100% O2 at 30°C were exposed for 5 min to hypoxia, then allowed to recover in oxygenated buffer alone or containing a single plant extract (0.001–1 mg/mL). The contractility (g/s) and beating frequency (beats/min), as well as troponin C contents of the bathing solution (ng/mL), were determined and expressed as means ± SDs.

Results The extracts of A. muricata, B. orellana, C. peltata and T. catappa caused an increase in the contractility compared to untreated atria of 340 ± 102%, 151 ± 13%, 141 ± 14% and 238 ± 44%, respectively. However, the latter two preparations increased the troponin C contents of the bathing solution to 36 ± 11 and 69 ± 33, compared to the value of 11 ± 3 ng/mL found with untreated atria.

Conclusions Preparations from A. muricata and B. orellana may possess positive-inotropic properties which may improve the contractility of the post-hypoxic myocardium. Studies to assess their usefulness in ischemic–reperfusion injury are warranted.

ARTICLE HISTORY

Received 13 May 2015
Accepted 8 October 2015
Revised 6 September 2015
Published online 31 December 2015

KEYWORDS

Hypoxia; troponin C; positive-inotropism

Introduction

Throughout the history of medicine, compounds with positive-inotropic properties have demonstrated their clinical usefulness for treating cardiovascular conditions requiring improvement of cardiac contractility such as congestive heart failure. Examples of such compounds are cardiac glycosides like digoxin (Campia et al. 2010), agonists of adrenergic and dopaminergic receptors like isproterenol and dobutamine (Kanter & DeBlieux 2014) and calcium sensitisers like levosimendan (Kolseth et al. 2014).

Some of these agents are also useful for improving the decreased cardiac contractility associated with ischemic–reperfusion injury (McMurray et al. 2012). Ischemic–reperfusion injury refers to damage occurring to the myocardium upon restoration of the coronary blood flow and the re-introduction of oxygen after a (relatively brief) period of ischemia (Frank et al. 2012). This condition is characterized by a decreased contractility of the heart muscle and is commonly seen during reperfusion in coronary artery heart disease and medical procedures involving temporary ischemia of myocardial tissue such as coronary artery bypass surgery (Frank et al. 2012).

As ischemic–reperfusion injury is accompanied by necrosis and apoptosis of myocardial cells besides cardiac contractile dysfunction, the mortality rate of patients undergoing coronary revascularization is substantial, ranging from 7% to 12% (Maharaj & Metaxa 2011). However, the use of the above-mentioned...
positive-inotropic agents to improve cardiac contractility in ischemic–reperfusion injury is often accompanied by serious adverse effects such as severe vasoconstriction of peripheral blood vessels, aggravation of heart failure or induction of cardiac arrhythmias (McMurray et al. 2012). Many plants contain constituents with presumed positive-inotropic effects. However, there is no or only little scientific evidence to support these suppositions. This also holds true for, for instance, *Abelmoschus esculentus* L. Moench (Malvaceae), *Annona muricata* L. (Annonaceae), *Bixa orellana* L. (Bixaceae), *Cecropia peltata* L. (Moraceae), *Erythrina fuscus* Lour. (Fabaceae), *Psidium guajava* L. (Myrtaceae) and *Terminalia catappa* L. (Combretaceae) (Table 1). These plants are popularly used as cardiotonic (Ambasta 1994; Raghoenandan 1994; DeFilipps et al. 2004), antispasmodic (DeFilipps et al. 2004), diuretic (Ambasta 1994) and against heart flutter (DeFilipps et al. 2004), which raises the possibility that they may possess meaningful positive-inotropic properties.

In the current study, the above-mentioned plants were evaluated for their potential to improve cardiac contractility in a laboratory model of ischemic–reperfusion injury. Thus, guinea pig atria were isolated, exposed for a relatively brief period to hypoxia, then reoxygenated, and subsequently assessed for improvement of their contractile response in the presence of extracts from the plants. As increased troponin C release is directly related to the extent of myocardial injury (McMurray et al. 2012), troponin C contents in the organ bath following exposure of the isolated atria to the plant extracts was also determined. The results with the plant extracts have been compared to those obtained in their absence and have been discussed in terms of the suitability of the plants to treat ischemic–reperfusion injury.

**Materials and methods**

**Plant collection and extraction**

The plants and plant parts used in this study as well as relevant details about them are listed in Table 1. They were collected in rural areas of Paramaribo, the capital city of Suriname, and authenticated by staff from the National Herbarium of Suriname by comparing with deposited voucher specimens. Collections were only carried out when it was certain that the collection areas had not been exposed to insecticides or other hazardous chemicals during the preceding six months. After washing with distilled water, the plant material was macerated and extracted with distilled water at 100 °C. The extracts were filtered, dried under reduced pressure at a temperature not exceeding 45 °C, and divided in aliquots of 3 g which were stored at −20 °C until testing. Typically, crude material weighing between 500 and 1000 g yielded 15–20 g of extract.

**Chemicals**

Norepinephrine was from Fluka AG (BuchsSG, Switzerland). All other chemicals used were from our laboratory stock and were of the highest grade available. The drugs and plant extracts were dissolved in an adapted Ringer-Locke buffer containing NaCl 5 g/L, KCl 0.4 g/L, CaCl$_2$ 0.24 g/L, NaHCO$_3$ 0.15 g/L and glucose 1 g/L. The final pH of the solution was adjusted to 7.4.

**Isolated guinea pig atria**

Guinea pigs were anesthetized with chloroform in a gassing chamber (25 °C). A parasternal incision was made to expose the heart and after dissection of the major blood vessels, the atria were quickly isolated in ice-cold buffer (<4 °C) and transferred to an organ bath containing 30 mL of Ringer-Locke solution kept at a temperature of 30 °C and gassed with pure oxygen. The tip of one auricle was attached to a fixed point in the bath, while that of the other was connected to an FT-100 force transducer (CB-Sciences, Dover, NH). The preload was gradually increased to a basic tension of 1.0 ± 0.1 g and kept at that value during the entire experiment. As soon as the operating temperature had been reached, the atria started to contract spontaneously. The atria were allowed to adjust to the experimental conditions for at least 20 min before the experiments were started. The buffer was regularly refreshed during that period. The entire experimental procedure had been approved by the Bioethics Committee of our institution (file number 1092A/13).

**Table 1. Relevant data about the plants used in this study.**

<table>
<thead>
<tr>
<th>Scientific name (vernacular name)</th>
<th>Voucher number</th>
<th>Plant family</th>
<th>Plant part used</th>
<th>Common use (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. esculentus L. Moench (Okra)</td>
<td>Uvs17504</td>
<td>Malvaceae</td>
<td>Dried seeds</td>
<td>Cardiotonic (Ambasta 1994)</td>
</tr>
<tr>
<td>A. muricata L. (Soursop)</td>
<td>Uvs17434</td>
<td>Annonaceae</td>
<td>Fresh leaves</td>
<td>Flutter of the heart (DeFilipps et al. 2004)</td>
</tr>
<tr>
<td>B. orellana L. (Annato)</td>
<td>Uvs17467</td>
<td>Bixaceae</td>
<td>Dried seeds</td>
<td>Cardiotonic (Raghoenandan 1994)</td>
</tr>
<tr>
<td>C. peltata L. (Trumpet tree)</td>
<td>Tva4868</td>
<td>Moraceae</td>
<td>Dried leaves</td>
<td>Cardiotonic (DeFilipps et al. 2004)</td>
</tr>
<tr>
<td>E. fuscus Lour. (Purple coral tree)</td>
<td>Th05</td>
<td>Fabaceae</td>
<td>Flower</td>
<td>Antispasmodic (DeFilipps et al. 2004)</td>
</tr>
<tr>
<td>P. guajava L. (Guava)</td>
<td>Uvs17462</td>
<td>Myrtaceae</td>
<td>Fresh leaves</td>
<td>Stomach cramps (DeFilipps et al. 2004)</td>
</tr>
<tr>
<td>T. catappa L. (Tropical almond)</td>
<td>Uvs17500</td>
<td>Combretaceae</td>
<td>Bark</td>
<td>Cardiotonic, diuretic (Ambasta 1994)</td>
</tr>
</tbody>
</table>
Experimental procedure

Before starting the experiments, the atria were stressed by exposing them for 2 × 3 min to hypoxia (by cutting off the oxygen supply and refreshing the bathing solution) and restoring the oxygen supply. After allowing the atria to recover for 6 min, experiments were started by exposing them for 5 min to hypoxia, after which they were reoxygenized and incubated for 6 min with fresh buffer alone, norepinephrine or a plant extract.

The resulting forces of contraction were registered by the force transducer and monitored with a desktop computer through an ETH-260 Bridge/Bio amplifier (CBSciences) connected to a Powerlab 400E analog/digital converter (ADInstruments, Castle Hill, Australia). Signals were recorded with the Chart for Windows 4.2.3 software (ADInstruments). The software also calculated and displayed the frequency of the contractions in beats/min, and generated the relative contractility dF/dt in g/s by differentiating forces of contraction in time.

The contractility and beating frequency of the reoxygenized atria in the presence of norepinephrine or the plant extracts were calculated at 90 s after reoxygenation and expressed relatively to values found with buffer alone.

Assessment of troponin C content

Troponin C contents of the bathing solution were determined from 1 mL aliquots taken at 90 s after reoxygenation and using a solid-phase enzyme-linked immunosorbent assay (NeoBiolab, Woburn, MA). Briefly, triplicate samples of 100 μL of the bathing solutions, along with triplicate samples of Ringer-Locke buffer and triplicate samples of calibration solution containing known concentrations of troponin C, were pipetted in 96-well microplates coated with specific anti-guinea pig troponin C monoclonal antibodies. Conjugate solution (50 μL) containing another anti-troponin C antibody and horseradish peroxidase was added. This resulted in the troponin C molecules being sandwiched between the solid phase and the horseradish peroxidase-linked antibodies.

After incubation for 60 min at 37°C, the wells were washed with distilled water to remove unbound-labelled antibodies. Tetramethylbenzidine solution (50 μL) was added for 15 min at room temperature, resulting in the development of a blue colour. The colour development was stopped with the addition of 50 μL HCl 1 N changing the colour to yellow. The optical density of the latter product was determined with a microplate reader at 450 nm and corrected for the optical density found with Ringer-Locke buffer alone. Using the optical densities obtained with the calibration solution, a dose–response curve was constructed from which troponin C contents of the samples were derived in ng/mL.

Statistics

The data presented are means ± SDs of at least three independent experiments performed in duplicate or triplicate. The results were analysed with one-way ANOVA and differences were considered statistically significant when p values < 0.05. The exact p values were calculated with Dunnett’s multiple comparison test.

Results

Effects of hypoxia and reoxygenation on the relative contractility of the isolated guinea pig atria

Figure 1 shows a representative recording of the relative contractility of untreated, oxygenized, spontaneously beating atria; during hypoxia and after reoxygenation. The spontaneous contractility was 3–4 g/s, and this value had decreased by 70–90% following 5 min of hypoxia. Reoxygenation led to a gradual recovery of the contractility, and initial values were reached after at least 4 min.

Effects of norepinephrine on the relative contractility and beating frequency of post-hypoxic isolated guinea pig atria and troponin C contents of the bathing solution

Figure 2(A) and (B) shows the relative contractility and beating frequency, respectively, of the post-hypoxic atria in the presence of norepinephrine at 90 s after reoxygenation. Both variables increased with increasing doses of norepinephrine, indicating that the use of this prototype positive-inotropic agent had improved the performance of the post-hypoxic and reoxygenated atria. Furthermore, troponin C contents of the bathing solution of the post-hypoxic atria exposed to norepinephrine 10 μM were 7 ± 3 ng/mL. This was in the same range as troponin C contents of the bathing solution of untreated spontaneously beating atria (6 ± 1 ng/mL), suggesting that the atria had recovered in the presence of norepinephrine without undergoing substantial damage. Together, these observations validated the use of the experimental set-up to evaluate plant extracts with presumed positive-inotropic properties for their ability to improve the performance of the post-hypoxic, reoxygenized myocardium.
Effects of plant extracts on the relative contractility and beating frequency of post-hypoxic isolated guinea pig atria

Table 2 gives the contractility of the post-hypoxic atria caused by the plant extracts at 90 s after re-oxygenation with respect to values found with buffer alone. Exposure of the post-hypoxic atria to the extracts from *A. muricata* (0.1 and 1 mg/mL), *B. orellana* (0.01 and 0.1 mg/mL), *C. peltata* (0.001, 0.001 and 0.1 mg/mL) or *T. catappa* (0.01 and 0.1 mg/mL) led to an increase in their contractility by 1.4- to 3.4-fold. These values were in the range of or even higher than the contractility found in the presence of norepinephrine at a concentration of at least $10^{-6}$ M (Figure 2A). The use of the *A. esculentus*, *E. fusca* or *P. guajava* extracts had no significant effect on this phenomenon. None of the plant extracts had a statistically significant effect on the beating frequency of the post-hypoxic atria (Table 3).

**Effects of hypoxia and plant extracts on troponin C contents of the bathing solution**

Troponin C contents of the bathing solution of hypoxic and untreated, reoxygenized (post-hypoxic) was slightly but statistically significantly higher than that of untreated, spontaneously beating atria ($13 \pm 3$ and
Table 2. Contractility of isolated guinea pig atria at 90 s after reoxygenation relative to untreated post-hypoxic atria (100%).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>0.001 mg/mL</th>
<th>0.01 mg/mL</th>
<th>0.1 mg/mL</th>
<th>1 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. esculentus</td>
<td>104 ± 20</td>
<td>129 ± 48</td>
<td>131 ± 54</td>
<td>114 ± 32</td>
</tr>
<tr>
<td>A. muricata</td>
<td>111 ± 16</td>
<td>126 ± 31</td>
<td>217 ± 65</td>
<td>340 ± 102a</td>
</tr>
<tr>
<td>B. oralana</td>
<td>109 ± 20</td>
<td>137 ± 19</td>
<td>151 ± 13</td>
<td>81 ± 34</td>
</tr>
<tr>
<td>C. peltata</td>
<td>135 ± 24a</td>
<td>126 ± 20a</td>
<td>141 ± 14</td>
<td>102 ± 45</td>
</tr>
<tr>
<td>E. fusca</td>
<td>103 ± 6</td>
<td>125 ± 17</td>
<td>123 ± 20</td>
<td>124 ± 11</td>
</tr>
<tr>
<td>P. guajava</td>
<td>110 ± 20</td>
<td>107 ± 15</td>
<td>117 ± 19</td>
<td>79 ± 36</td>
</tr>
<tr>
<td>T. catappa</td>
<td>187 ± 35</td>
<td>221 ± 15*</td>
<td>238 ± 44</td>
<td>167 ± 82</td>
</tr>
</tbody>
</table>

*Significantly different from control (p < 0.05; Dunnett’s multiple comparison test).

Table 3. Beating frequency of isolated guinea pig atria at 90 s after reoxygenation relative to untreated post-hypoxic atria (100%).

<table>
<thead>
<tr>
<th>Plant species</th>
<th>0.001 mg/mL</th>
<th>0.01 mg/mL</th>
<th>0.1 mg/mL</th>
<th>1 mg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. esculentus</td>
<td>89 ± 13</td>
<td>114 ± 24</td>
<td>115 ± 23</td>
<td>102 ± 25</td>
</tr>
<tr>
<td>A. muricata</td>
<td>105 ± 3</td>
<td>103 ± 9</td>
<td>117 ± 7</td>
<td>122 ± 9</td>
</tr>
<tr>
<td>B. oralana</td>
<td>100 ± 2</td>
<td>115 ± 19</td>
<td>114 ± 16</td>
<td>70 ± 30</td>
</tr>
<tr>
<td>C. peltata</td>
<td>101 ± 9</td>
<td>102 ± 4</td>
<td>99 ± 11</td>
<td>86 ± 17</td>
</tr>
<tr>
<td>E. fusca</td>
<td>93 ± 8</td>
<td>107 ± 7</td>
<td>128 ± 53</td>
<td>111 ± 40</td>
</tr>
<tr>
<td>P. guajava</td>
<td>98 ± 8</td>
<td>97 ± 6</td>
<td>99 ± 14</td>
<td>93 ± 27</td>
</tr>
<tr>
<td>T. catappa</td>
<td>87 ± 13</td>
<td>103 ± 3</td>
<td>99 ± 7</td>
<td>103 ± 15</td>
</tr>
</tbody>
</table>

11 ± 3 ng/mL, respectively, versus 6 ± 1 ng/mL; Table 4). These observations suggest that the cardiomyocytes of hypoxic and untreated post-hypoxic atria had suffered relatively little damage.

The addition of the extracts from A. esculentus (1 mg/mL), A. muricata (1 mg/mL), B. oralana (0.1 mg/mL), E. fusca (1 mg/mL) or P. guajava (1 mg/mL) to the post-hypoxic atria led to troponin C values that did not differ statistically significantly from those found in their absence (9 ± 3 to 24 ± 12 ng/mL; Table 4). However, troponin C contents of the bathing solution of post-hypoxic atria treated with the C. peltata (0.1 mg/mL) or T. catappa extract (0.1 mg/mL) had increased to 36 ± 11 and 69 ± 33 ng/mL, respectively. These observations suggest that the two latter plant extracts, in contrast to the former three, had inflicted substantial injury to the cardiomyocytes.

Discussion

In this study, seven Surinamese plants with presumed positive-inotropic properties were evaluated for their potential to improve the decreased contractility of post-hypoxic, reoxygenized isolated guinea pig atria. Our results show that in the presence of the extracts from A. muricata (leaves) and B. oralana (seeds) the relative contractility of the atria was improved, while leaving the beating frequency unaffected and slightly raising troponin C contents in the organ bath. The contractility of the atria was also increased in the presence of the extracts from C. peltata (leaves) and T. catappa (bark) without affecting the beating frequency. However, this was accompanied by a substantial increase in troponin C contents of the bathing solution. The extracts from A. esculentus (dried seeds), E. fusca (bark) and P. guajava (leaves) showed no effect on either the contractility or the beating frequency of the post-hypoxic isolated atria and raised troponin C contents in the bathing solution only to some extent. Together, these observations suggest that the extracts from A. muricata and B. oralana had elicited appreciable positive-inotropism while affecting myocyte viability only marginally, that those from C. peltata and T. catappa had positive-inotropic effects but also caused damage to the myocytes, and that the A. esculentus, E. fusca and P. guajava preparations did not affect either phenomenon.

Partial support for the positive-inotropic effects seen with the A. muricata preparation comes from the increased contraction force of isolated frog hearts caused by an aqueous extract from fresh leaves of Annona squamosa L. (Sherikar et al. 2010); the increased contractility of isolated rat atria (Praman et al. 2013) and the increased systolic pressure in human subjects (Lee et al. 2013) induced by the alkaloid higenamine, an active constituent of this plant (Wagner et al. 1980) and the positive-inotropic effect of higenamine in isolated murine atria through stimulation of adrenergic receptors (Kimura et al. 1994). However, higenamine was also reported to exert positive-chronotropism, and its presence in A. muricata has yet to be determined. Still, the relatively low troponin C release by the post-hypoxic guinea pig atria exposed to the A. muricata extract noted in the current study, supports the ethnopharmacological use of this preparation against...
cardiac flutter (DeFilipps et al. 2004) and justifies its further evaluation in at least preclinical models of ischemic–reperfusion injury.

The same may hold true for the preparation from B. orellana that also displayed positive-inotropism in the current study without substantially affecting beating frequency and troponin C release. Notably, in a previous study (Asokkumar & Jagannath 2012), pretreatment with an ethanol extract from the fruits from this plant protected laboratory rats from myocardial necrosis induced by isoproterenol-induced infarction. This effect had been attributed to the anti-oxidant properties of this preparation and was comparable to that elicited by the anti-oxidant a-tocopherol (Asokkumar & Jagannath 2012). However, in light of its ethnopharmacological use as a cardiotonic (Raghoeinanand 1994), more detailed studies about its presumed positive-inotropism should be carried out.

The apparent positive-inotropic properties of the C. peltata and the T. catappa extracts seen in the current study are in line with those reported for several Cecropia species in isolated rat hearts (Consolini et al. 2006); the bronchodilation caused by an aqueous extract of Cecropia glaziovii Sneth in guinea pigs challenged with histamine (Delarcina et al. 2007); the apparent involvement of adrenergic stimulation in the latter observation (Delarcina et al. 2007); and the beneficial effect of an aqueous extract from the bark of Terminalia arjuna (Roxb) Wight & Arn on myocardial contractility in rabbit hearts exposed to ischemic–reperfusion (Gauthaman et al. 2005) and human subjects suffering from heart failure (Radhakrishnan et al. 1993). These findings support the use of these plants as cardiotonics and diuretics in traditional medicine (Ambasta 1994; DeFilipps et al. 2004).

On the other hand, preparations from the leaves of Urtica dioica L. and Combretum molle R Br ex G Don, other members of the plant families Moraceae and Combretaceae, respectively, have been associated with substantial cardiotoxicity and irreversible acute hypotension in laboratory rats (Tahri et al. 2000), as well as considerable toxicity in isolated guinea pig atria (Tahri et al. 2000), respectively. These findings may explain, at least partially, the relatively high troponin C levels observed in the current study in the bathing solution of post-hypoxic guinea pig atria treated with the C. peltata or the T. catappa extract. Obviously, this supposition must be verified in future studies but if true, it precludes the potential usefulness of C. peltata and T. catappa preparations as positive-inotropic compounds in ischemic–reperfusion injury.

The absence of an effect of the extracts from A. esculentus, P. guajava and E. fusca on relative contractility and beating frequency of, and troponin C release by post-hypoxic isolated guinea pig atria suggests that these preparations do not possess meaningful positive-inotropic properties. This also prohibits their potential use in ischemic–reperfusion injury. However, these preparations may influence cardiac performance through mechanisms other than those directly affecting myocardial contractility. Indications for this supposition are provided by the vasodilating effects in laboratory rats of quercetin and hyperin found in the leaves of P. guajava and the flowers of Abelmoschus species, respectively (Matsuzaki et al. 2010; Fan et al. 2011; Xue et al. 2011), and the anxiolytic effect in mice of the tetrahydroisoquinoline alkaloid erytravine present in Erythrina species (Flausino et al. 2007). These mechanisms could lead to an increase in cardiac output following reduction of the afterload, and may explain the folk medicinal use of these preparations as cardiotonic, antitussive and spasmylocytic substances, respectively (Ambasta 1994; DeFilipps et al. 2004).

Conclusions

Aqueous extracts from parts of A. muricata and B. orellana seem to possess positive-inotropic properties that may improve myocardial performance in ischemic–reperfusion injury without causing damage to the myocytes. Those from the other plants either may cause excessive toxicity to the myocytes (C. peltata and T. catappa) or do not display positive-inotropic characteristics (A. esculentus, P. guajava and E. fusca). Future studies with laboratory animals should verify the potential usefulness of preparations from A. muricata and B. orellana in the treatment of ischemic–reperfusion injury.

Declaration of interest

This study was partly funded by the Research and Development Fund of the Anton de Kom University of Suriname, the VLIR-IUC, and the Suriname Conservation Foundation. The authors declare that there is no conflict of interest regarding the publication of this paper.

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