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Aqueous extract from leaf of *Artocarpus altilis* provides cardio-protection from isoproterenol induced myocardial damage in rats: Negative chronotropic and inotropic effects

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ABSTRACT

Ethnopharmacological relevance: The leaves of *Artocarpus altilis* (Parkinson ex F.A.Zorn, Fosberg) (Moraceae) are used in the management of hypertension; this study assessed the cardio-protective effects of the leaf extract on isoproterenol (ISO) induced myocardial damage in rats.
Material and methods: Twenty (20) adult male Sprague-Dawley rats (175–230 g) were divided into 5 groups. Group 1 (Control), 2 (AA) received 50 mg/Kg *Artocarpus altilis* (AA) only; 3 (ISO) received 85 mg/Kg ISO only; 4 (ISO+AA/50) and 5 (ISO+AA/100) received 50 and 100 mg/Kg AA respectively for 6 days, after induced with ISO twice (85 mg/Kg) at a 24-h period. Blood pressure readings were taken before and after the administering of ISO using the tail cuff method. ECG was performed on anaesthetized rats. Cardiac contractility was measured in isolated right atrial muscles. Assessment of myocardial infarct (MI) size, heart/body weight ratio, biochemical, hematological and histo-morphological parameters were conducted at the end of seven days. An aqueous extract from leaves of *A. altilis* was analyzed for organic compounds using UHPLC mass spectrometry.
Results: ISO induced myocardial damage through an elevation of the heart rate (HR), infarct size and ECG distortions. Treatment with AA significantly ($p<0.05$) reduced heart/body weight ratio (49%), MI (96%), HR (27%), sympathovagal imbalance (36%) and serum cardiac biomarkers (AST, LDH, HDL, triglycerides and CCK) caused by ISO. AA decreased the beat frequency of isolated right atrium (11%) cause by ISO, an action similar to propranolol (beta-adrenergic antagonist; 20%), but showed no significant changes in the QTc intervals of the ECG (suggesting no cardio-toxic drug-herb interactions), Thirty nine compounds were detected using high resolution LC-MS analysis (HPLC-Orbitrap-APCI-MS) in the extract. Pure compounds, as gallic acid and rutin, presented a higher negative chronotropic effect, similar to propranolol.
Conclusion: Oral administration of aqueous extract of *Artocarpus artilis* has cardio-protective functions in myocardial injury, in part, by decreasing the HR, reduced contractility and infarct size. These findings may explain the cardio-protective use of *A. altilis* in traditional medicine.

1. Introduction

Breadfruit (*Artocarpus altilis*, Parkinson ex F.A.Zorn, Fosberg) is a species of flowering tree in the mulberry (Moraceae) family. In the Caribbean, extracts from these leaves are used as cardiac tonics, to strengthen the heart and for the treatment and management of hypertension (Lans, 2006; Siddesha et al., 2011; Young et al., 1993). Infusions from this plant species have been reported to reduce high blood pressure by direct cardiac adrenergic activation, activation of vascular endothelium, and spasmolytic effect on vascular smooth

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muscle via Ca^{2+} channel antagonism (Nwokocha et al., 2012). These leaves extract exerts antioxidant, anti-inflammatory and angiotensin-converting-enzyme (ACE) inhibitor activity through its glycosidic, phenolic, prenylated flavonoids and γ-aminobutyric acid contents (Siddesha et al., 2011). They also possess a weak, negative chronotropic effect and significantly reduced left ventricular pulse pressure due to the presence of ethyl acetate (Young et al., 1993).

Cardiovascular disease complications manifest in various forms and may lead to lethal outcomes in myocardial infarctions (Bolli, 1994). Acute myocardial infarction (AMI) is considered one of the leading causes of death in the world among humans and is often associated with hypertension. Due to the high cost of medications and the prevalent poverty burden, various traditional and folkloric approaches have been adopted such as the use of some medicinal plants (Nwokocha et al., 2012). ISO is a potent nonselective beta-adrenergic agonist, known to induce myocardial infarction at high doses. It is hypothesized to cause myocardial infarction through auto-oxidation and generation of free cytotoxic radicals (Singal et al., 1983), as well as hyper-stimulation of the beta adrenoceptors (Haenen et al., 1990). These actions will lead to the peroxidation of the cellular membrane, a change in the membrane permeability and possibly derangement of calcium ion pathway signaling and myocardial injury (Tappia et al., 2001).

To the best of our knowledge, the direct effect of aqueous leaf extract of *Artocarpus altilis* on ISO-induced myocardial injury has not been previously examined. This study is designed to study the cardio-protective properties of *A. altilis*, and the possible mechanism(s) of action, using *in vivo* and *in vitro* methods of normotensive and myocardial injury models. In this work we have also analyzed the aqueous leaf extract of leaves from this important tropical fruit using state of the art high resolution mass spectrometry (UHPLC-Q-Orbitrap-MS/MS) (Simirgiotis et al., 2016b) in order to rapidly detect its constituents.

2. Material and methods

2.1. Plant material and extraction

Leaves of *A. altilis* were collected in February and botanical identification of the plant was made by Mr. Patrick Lewis, Department of Botany, University of The West Indies, Mona Campus and a voucher specimen AN 08, 10/11 (for UWI), and Nr-111004-1 (for Chile) of the plant material deposited in each department. The collected leaves of *A. altilis* were shade dried (25 °C) and ground into coarse powder. The powdered leaves were macerated with warm distilled water and left to stand overnight. The extracts was filtered using filter paper (Whatman), evaporated to dryness to yield a dark brown residue with a 29.2% yield. The concentrated extract was stored in a capped container and refrigerated at −4 °C until ready for use.

2.2. Experimental animals

The study used male Sprague Dawley rats (8–10 weeks old) weighing between 170 g and 230 g and was conducted in accordance with the Animal Scientific Procedures Act of 1986 following ethical approval from the UWI/FMS Ethics Committe (AN 06,15/16). The animals were housed in plastic cages at a room temperature of 22–25 °C and humidity of 45–51% and they had access to tap water and food *ad libitum*. Rats were randomly assigned into five groups of 4 animals each. Group 1 served as the control, group 2 received 50 mg/Kg *Artocarpus altilis* liquid extract only, while group 3 received 85 mg/Kg ISO only. Groups 4 and 5 received 50 and 100 mg/Kg *Artocarpus altilis* extract by gavage respectively for 6 days, after being induced with ISO twice (85 mg/ Kg) twice during a 24 h period intraperitoneally (i.p.). At the end of the treatment period animals were sacrificed on the 7th day under anesthesia (diethyl ether). In this study we used the lowest number of animals in each group as suggested by our Ethics Committee and international regulations and ethical considerations, but is necessary to note that it could be a limitation of the results of this study and should be repeated later using a higher number of animals.

2.3. Blood pressure and ECG recording

Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) were measured using the tail cuff method (CODA) before and after the administration of ISO. Pulse pressure (PP) was calculated using the formula: PP =(SBP - MBP). Mean Arterial Pressure (MAP) was calculated using the formula: $MAP = P_{diastole} + 1/3 (P_{systole} - P_{diastole})$. For ECG recordings, briefly, rats were first anesthetized with 100 mg/Kg Ketamine and 10 mg/Kg Xyline intra-peritoneally (i.p). The ECG electrodes were placed subcutaneously (with syringes) in bipolar configuration (DII). Measurements were done using the ECG100C (BIOPAC) Electrocardiogram Amplifier equipment and tracings were recorded using the AcqKnowledge III computer software program. The QT interval is taken as the time from the beginning of the QRS complex to the end of the T wave. The RR interval is taken as the time elapsed between two consecutive maxima of the R waves. The corrected QT interval (QTc) was calculated in accordance with the formula: $QTc = QT/(RR)^{1/2}$ (Cifuentes et al., 2016). The frequency bands used were: total power (P: 0–3 Hz), power in the low-frequency (LF: 0.20–0.75 Hz), high-frequency (HF: 0.75–3.0 Hz). Sympatovagal balance was calculated as the LF to HF ratio (LF/HF) (Cifuentes et al., 2016).

2.4. Frequency and contractility of isolated right atrium of the rat

For this procedure, the heart was quickly excised after sacrifice, and placed in a cold (4 °C) physiological Krebs-Ringers buffer (KRB) containing (in mM): 4.2 KCl, 1.19 KH_2PO_4, 120 NaCl, 25 Na_2HCO_3, 1.2 $MgSO_4$, 1.3 $CaCl_2$, and 5 D-glucose (pH 7.4; 37 °C; 95% O_2 and 5% CO_2). The rat isolated right atrium was carefully fixed with silk thread into the organ bath. The lower one was attached to a stationary glass rod and the upper one was attached to an isometric transducer (Radnoti, Monrovia, California). The transducer was connected to a PowerLab 8/35 (Colorado Springs CO) for continuous recording of vascular tension using the LabChart Pro v8.1.2 computer program (ADSInstrument). The passive tension on right atrium was 0.5 g. Cumulative concentrations of *Artocapus altilis* (0–1000 g/mL) or different metabolites (10^{-7} to 10^{-4} M) were added to the medium every 5 min.

2.5. Blood parameters and biochemical estimation

Blood samples were collected and placed into standardized EDTA tubes for complete blood count. Hematological parameters were assessed using the coulter counter. The serum was separated and preserved at −20 °C for estimation of the biochemical parameters. High-density lipoprotein (HDL), creatinine phosphor kinase (CPK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), cholesterol and triglycerides (TGs) were measured using the Next-generation cobas 6000 Analyzer Series Automated Analyzer Roche Diagnostics, North America.

2.6. Determination of myocardial infarct size and histo-morphological appraisal

After the rats were sacrificed from each group, the heart tissues were harvested and immediately submerged in 10% neutral buffered formalin for preservation. They were subsequently processed, embedded in wax, and serial sectioned to a thickness of 4 μm then stained with haemotoxylin and eosin (H & E) stain. Twenty (20) full-thickness transverse sections from the heart, immediately subjacent to the atrio-ventricular valves (four from each group), were analyzed and evaluated