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Clinical efficacy of water extract of stem bark of *Terminalia arjuna* (Roxb. ex DC.) Wight & Arn. in patients of chronic heart failure: a double-blind, randomized controlled trial

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ABSTRACT

Background: The stem bark of *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn. (*Arjuna*) is used in Indian system of medicine (*Ayurveda*) for treatment of various cardiac diseases, including heart failure. However, well designed clinical trials exploring its efficacy and safety in chronic heart failure (CHF) are lacking.
Purpose: To ascertain the add-on efficacy and safety of a standardized water extract of stem bark of *Arjuna* (*Arjuna* extract) in CHF patients on standard pharmacotherapy.
Study design: Double-blind, parallel, randomized, placebo-controlled add-on clinical trial.
Methods: After approval of institutional ethics committee, 100 patients of CHF of New York Heart Association (NYHA) functional class II on standard pharmacotherapy having an echocardiographic left ventricular ejection fraction (LVEF) $\leq$ 40% were consecutively recruited with informed consent and randomized 1:1 to *Arjuna* extract 750 mg or matching placebo twice daily. The primary outcome measure was change in LVEF at 12 weeks. Secondary outcome measures included changes in (i) NYHA functional class, (ii) distance covered in 6 min walk test (6MWT), (iii) quality of life (QoL), as determined by the Kansas City Cardiomyopathy Questionnaire (KCCQ), (iv) plasma brain natriuretic peptide, (v) plasma cytokines (interleukin-6, high sensitivity C-reactive protein and tumour necrosis factor-α) and (vi) oxidative stress markers [serum thiobarbituric acid reactive substances (TBARS), red blood cell (RBC) superoxide dismutase (SOD), RBC catalase and RBC glutathione (GSH)] at 6 and 12 weeks. Safety assessment was done by adverse event monitoring and laboratory investigations. Results were expressed as mean $\pm$ SD or median (interquartile range) and analysed with intention-to- treat principle using appropriate two-sided statistical tests. A p-value < 0.05 was considered significant.
Results: *Arjuna* extract was well-tolerated, but did not change LVEF (24.3 $\pm$ 7.1 versus 25.5 $\pm$ 7.7%; $p = 0.4$) or secondary outcome measures except preservation of RBC catalase activity [1275(104, 10350) versus 1243.5(104, 10350) U/g haemoglobin; $p = 0.01$] compared to placebo. Significantly greater percentage increases occurred in distance covered in 6 MWT, RBC-SOD, RBC catalase, RBC GSH and in symptom severity and stability domains of KCCQ in patients on *Arjuna* extract versus those on placebo, on a post-hoc analysis, between subgroups of patients who improved in these outcomes.
Conclusion: *Arjuna* extract did not improve LVEF in CHF patients over 12 weeks, although there was improvement in functional capacity, antioxidant reserves and symptom-related QoL domains in some patients.

Abbreviations: 6MWT, 6 minute walk test; ACEI, angiotensin converting enzyme inhibitor; AE, adverse event; ANCOVA, analysis of covariance; API, Ayurvedic Pharmacopoeia of India; ARB, angiotensin II type 1 receptor blocker; BNP, brain natriuretic peptide; CDSCO, Central Drugs Standard Control Organization; CHF, Chronic heart failure; DLC, differential leukocyte count; ECG, electrocardiography; EDTA, ethylenediaminetetraacetic acid; ESR, erythrocyte sedimentation rate; FBS, fasting blood sugar; GSH, glutathione; Hb, haemoglobin; hsCRP, high sensitivity C-reactive protein; IL-6, interleukin-6; IQR, interquartile range; IU, International Units; KCCQ, Kansas City Cardiomyopathy Questionnaire; LV, left ventricular; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; PPBS, postprandial blood sugar; QoL, quality of Life; RBC, red blood cell; S. ALP, serum alkaline phosphatase; S. Bil., serum bilirubin; S. HDL-C, serum high density lipoprotein cholesterol; S. LDL-C, serum low density lipoprotein cholesterol; SAE, serious adverse event; SD, standard deviation; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; SOD, superoxide dismutase; T_3, triiodothyronine; T_4, thyroxine; TBARS, thiobarbituric acid reactive substances; TLC, total leukocyte count; TNFα, tumour necrosis factor-α; TSH, thyroid stimulating hormone; V0, baseline visit; V1, 6th week visit; V2, 12th week visit.

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Introduction

Chronic heart failure (CHF) is a common disorder afflicting humans, more so in the elderly. Despite considerable advances made in the last 4 decades, the mortality and morbidity in heart failure patients is still substantial. Evidence-based pharmacotherapy has improved survival rates in patients of chronic heart failure with a reduced ejection fraction (McMurray et al. 2012). However, they continue to experience a poor quality of life due to relentless disease progression and side effects of the drugs. Several drugs used in the treatment of various diseases including heart failure were originally isolated from natural sources, e.g. digoxin. The quest for newer therapeutic modalities, therefore, takes mankind back to nature.

The stem bark of *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn. (*Arjuna*) (name verified at http://www.theplantlist.org) is widely used in Indian system of medicine (*Ayurveda*) for various cardiovascular ailments (Dwivedi and Chopra 2014). It is an evergreen tree of Combretaceae family, popularly known as *Arjuna* in many Indian languages and is widely found in the Indian subcontinent. The bark has been reported to contain several bioactive compounds e.g. triterpenoids, glycosides and flavonoids (Dwivedi and Chopra 2014). Many experimental studies have reported its positive inotropic (Radhakrishnan et al. 1993), anti-ischemic (Dwivedi et al. 1988), antihypertensive (Takahashi et al. 1997), anti-hypertrophic (Kumar et al. 2009) and antioxidant (Dwivedi et al. 1988; Pawar and Bhutani 2005) effects, which have been reviewed extensively (Dwivedi and Chopra 2014; Maulik and Katiyar 2010). All these effects have therapeutic relevance in cardiovascular diseases of humans.

Clinical studies have reported its efficacy in various cardiovascular diseases (Maulik and Talwar 2012), mostly in patients with ischemic heart disease (Bharani et al. 2002; Dwivedi and Agarwal 1994), hypertension (Rao et al. 2001) and heart failure (Bharani et al. 1995). However, well designed randomised clinical trials of a standardised extract of *Arjuna* in heart failure are still lacking. Therefore, the present study was designed to explore the add-on efficacy and safety of a standardized aqueous extract of stem bark of *Arjuna* (*Arjuna* extract) in chronic heart failure patients with reduced ejection fraction already on standard pharmacotherapy.

Methods

The aqueous extract of stem bark of *Terminalia arjuna* (Roxb. ex DC.) Wight and Arn., was prepared, standardized and formulated by Dabur India Limited, India on the directions of the study sponsor, the Department of Biotechnology, Government of India. The stem bark obtained from Madurai district, Tamil Nadu, India, during the months of September–October, was authenticated botanically by Dabur India Limited, in accordance with the Ayurvedic Pharmacopoeia of India (API) and a sample preserved as voucher specimen no. R&D/AYU/STD/003. The powder obtained from pulverising the dried stem bark was extracted by hot continuous percolation with 100% double distilled water for 72 h. The extract was then filtered and lyophilised to a powder. Ratio of herbal drug to extract was 20:1. The extract prepared in accordance with API is licensed in India to be used in cardiac disorders by practitioners of *Ayurveda* (Department of AYUSH 2011).

Qualitative tests were done to confirm the presence of glycosides, flavonoids, polyphenols, saponins and terpenoids (Kumar et al. 2009). The water extract was standardized with respect to the marker compound Arjunetin (0.10 ± 0.02% w/w), using High Performance Thin Layer Chromatography (HPTLC) carried out at the Analytical Development Laboratory, Dabur India Limited, India according to a widely used method (Varghese et al. 2015). Arjunetin, used as reference standard, was procured from Natural Remedies, Bangalore, Karnataka, India. The method for performing this analysis was as follows: HPTLC system consisted of Linomat 5 automated applicator [CAMAG] and photo documentation was done by CAMAG TLC visualizer and WinCATS ver. 1.4.6 scanner [CAMAG]. After filtration through 0.2 μm membrane filter, sample and standard were applied to the silica gel 60 F_{254} plate as bands of 6 mm. The solvent system consisted of ethyl acetate: toluene: formic acid: acetic acid = 6:3:0.5:1 [v/v/v/v] and detected at 690 nm after derivatization with anisaldehyde sulphuric acid spray reagent. The mobile phase was allowed to rise up to 8 cm. The analysis was repeated thrice by comparing and interpolating the extract peak area (response) with that of standard Arjunetin from the calibration curve. The quantity of Arjunetin per unit dosage form was standardized to be 0.75 ± 0.02 mg.

The amounts of microbial and heavy metal contaminants observed were within the limits specified in the API (Department of AYUSH 2011). This standardised aqueous extract of *Arjuna* and placebo (lactose) were formulated as matching hard gelatin capsules by Dabur India limited, India. Preclinical studies to determine the safety of the human equivalent dose of the extract were done at B.V. Patel Pharmaceutical Education and Research Development (PERD) centre, Ahmedabad, Gujarat, India as per ICH guidelines.

A double-blind, parallel, randomized, placebo-controlled clinical trial was conducted to ascertain the add-on efficacy and safety of *Arjuna* extract to evidence-based pharmacotherapy in chronic heart failure patients of New York Heart Association (NYHA) class II functional status at the heart failure clinic of the All India Institute of Medical Sciences (A.I.I.M.S.), New Delhi. Ethical approval was granted by the Institutional ethics committee of A.I.I.M.S., New Delhi. The trial was registered with the clinical trials registry of India (CTRI/2010/091/000415) (www.ctri.nic.in). The study followed the guidelines of the Declaration of Helsinki and Tokyo for humans. All subjects gave written informed consent to participate in the study.

The study consecutively recruited 100 (i) ambulatory chronic heart failure patients, (ii) of both genders, (iii) of age ≥ 18 years, (iv) having a left ventricular ejection fraction (LVEF) ≤ 40% on 2D-echocardiography with (v) NYHA functional class II symptoms for the past 3 months, (vi) receiving standard medical therapy, including an angiotensin converting enzyme inhibitor (ACEI) or an angiotensin II type 1 receptor blocker (ARB) and a beta-blocker as tolerated with/without a diuretic and digoxin. They were randomized 1:1 to receive either capsule of *Arjuna* extract 750 mg or matching placebo twice daily for 12 weeks, a dosing regimen providing the total daily dose of *Arjuna* extract used in a previous study (Bharani et al. 1995). The study excluded patients who had: (i) history of myocardial infarction or unstable angina (in the preceding 4 weeks), (ii) stroke or transient ischemic attack (in the preceding 6 months), (iii) cardiac revascularization procedures (in the preceding 3 months) or a planned cardiac revascularization (in succeeding 3 months), (iv) NYHA functional class III or IV symptoms, (v) primary valvular heart disease, (vi) evidence of myocarditis, aortoarteritis or coarctation of aorta (vii) uncontrolled hypertension (BP > 160/110 mm of Hg), (viii) hypothyroidism (serum TSH > 4.1 μIU/ml), (ix) liver dysfunction (serum bilirubin > 1.5 times upper limit of normal or serum transaminases > 3 times upper limit of normal), (x) renal dysfunction (serum creatinine > 1.5 mg/dl), (xi) pregnancy, lactation or women having child bearing potential not practising adequate contraceptive measures or (xii) consumed any other investigational drug or enrolled in any other study.

Outcome measures

The primary outcome measure was change in left ventricular ejection fraction (LVEF) measured using 2D-echocardiography at 12 weeks of treatment. Secondary outcome measures included