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Food Science and Human Wellness 2 (2013) 29–38

Food Science and Human Wellness

www.elsevier.com/locate/fshw

Protective role of concomitant administration of flax lignan concentrate and omega-3-fatty acid on myocardial damage in doxorubicin-induced cardiotoxicity

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Received 12 October 2012; received in revised form 19 December 2012; accepted 10 January 2013

Abstract

The severe cardiotoxicity incurred due to doxorubicin limits the use of their therapeutic potential. The current study aims to investigate the cardioprotective effect of concomitant administration of flax lignan concentrate (FLC) and omega-3-fatty acid (ω-3-FA) on myocardial damage in doxorubicin-induced cardiotoxicity in Wistar rats. Cardiotoxicity was induced by intraperitoneal injection of doxorubicin (4 mg/kg) on day 7th, 14th, 21st and 28th day in normal saline. Concomitant administration of FLC (500 mg/kg) and ω-3-FA (1 mL/kg) lowered TNF-α level, normalized ST, QT and mean arterial blood pressure, elevation in endogenous enzymes levels such as glutathione and lowering in malondialdehyde, super oxide dismutase followed by normalized lipid profile and reduced the mortality rate. The treatment had antiapoptotic potential at cellular level also histopathology of heart tissue (light and electron microscopical). Thus concomitant action of FLC and ω-3-FA may be antioxidant, antihyperlipidemic, anti-inflammatory and anti-apoptotic actions seem to the probable mechanisms in doxorubicin induced cardiotoxicity. It can be concluded that FLC and ω-3-FA both have distinct mechanism for cardioprotection and hence the additive effect was observed in the present study due to concomitant administration of FLC and ω-3-FA.

Keywords: Apoptosis; Cardiotoxicity; Doxorubicin; Flax lignan concentrate; Poly unsaturated fatty acid; TNF-α

1. Introduction

Flaxseed has been used as human food for thousands of years; industrial uses of flax oil have predominated since the industrial revolution. There has been considerable interest in the inclusion of flaxseed in western diet, medicinal food or in the development of isolated and purified compounds for food, animal feed, and the improvement of human health. Flaxseed seed with its high level of omega-3-fatty acid (ω-3-FA), assumes super food status. But due to the presence of several antinutrients such as cynogenic glycosides, linatine anti-vitamin B6, flaxseed is not generally regarded as commonly edible food [1]. An array of therapeutic activities of flaxseed is well proven one of them is antiatherosclerotic activity which is mainly attributed to secoisolariciresinol diglucoside (SDG) [2]. In our previous study, we have reported the cardioprotective activities in isoprenaline induced cardiotoxicity of flax lignan concentrate (FLC) extracted from flaxseed [3] and *in vitro* antioxidant activity of SDG lignan is previously reported [4].

For many years, scientists were puzzled by the fact that heart disease among Greenland Eskimos was extremely rare

Abbreviations: SDG, secoisolariciresinol diglucoside; FLC, flax lignan concentrate; ω-3-PUFA, omega-3-polyunsaturated fatty acids; CVD, cardiovascular disease; DHA, docosahexanoic acid; Dox, doxorubicin; ω-3-FA, omega 3 fatty acid; TG, triglycerides; TC, total cholesterol; HDL-C, high density lipoprotein; VLDL, very low density lipoprotein; SOD, super oxide dismutase; MDA, malondialdehyde; GSH, hlutathione; i.p., intrapritoneally; HR, heart rate; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MABP, mean aterial blood pressure; ELISA, enzyme-linked immunosorbent assay; ROS, reactive oxygen species; TNF-α, tumor necrotic factor-α level; ECG, electrocardiographic; TEM, transmission electron microscope.

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Peer review under responsibility of Beijing Academy of Food Sciences.

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http://dx.doi.org/10.1016/j.fshw.2013.01.002

despite their consumption of a high fat, high-cholesterol diet. Later research revealed that the Eskimos were protected by diets largely based on seals, whales and fish, all of which provide high intakes of omega-3 polyunsaturated fatty acids (ω-3-PUFA), especially eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) resourced from marine origin [5]. This observation established "Eskimo paradox" wherein little evidence of heart disease and low blood cholesterol levels was reported [6]. Now it is well established that there is an inverse relationship between high fish oil consumption and mortality following cardiovascular disease (CVD). The mechanism by which ω-3-FA may reduce risk for cardiovascular disease includes reduction of susceptibility of the heart to ventricular arrhythmia, antithrombogenic, hypotriglyceridemic, retardation of growth of atherosclerotic plaque, reduction of adhesion molecule expression, reduction of platelet-derived growth factor, anti-inflammatory, promotion of nitric oxide-induced endothelial relaxation and mild hypotension [7].

Despite of controversy about the mechanism of doxorubicin (Dox) on tumor cells, doxorubicin induced cardiotoxicity is a well established model to study the cardiovascular effects of different natural and synthetic drugs in rodents. Various mechanism of doxorubicin toxicity in rodents has been suggested. Doxorubicin administration increased oxidative stress due to production free radicals and caused cardiac deficit of antioxidants. At the nuclear level, intercalation into DNA, leading to inhibition of synthesis of macromolecules has been suggested, which precipitate DNA abnormalities like alkylation, crosslinking, strand separation and helicase activity. DNA damage may be initiated *via* inhibition of topoisomerase II and induction of apoptosis in response to topoisomerase II inhibition [8]. There are several reports of reversal of doxorubicin cardiotoxicity by natural products [9] including fish oil containing ω-3-FA [10], apart from this model has been successfully used in evaluation of cardioprotective action of number of extracts from medicinal plants such as *Zingiber officinale* [11], garlic [12].

However, different types of events, such as anti-inflammatory, anti-atherosclerotic and anti-immunomodulatory effects, have not yet been well explained and further focused studies are required to explore these properties to establish an optimized conventional drug therapy for cardioprotection [13]. Several reports have been published, emphasizing that statins alone are not sufficient and the use of combination therapy with ω-3-FA and statin is recommended for cardiovascular prognosis [14]. Nadtochiy and Redman [15] reported a very systematic approach for cardioprotective effects of the Mediterranean diet explained, which is due to PUFA and different types of polyphenol, they possibly act through different, distinct and convergent mechanisms. These findings explain the need for a novel design for the treatment of cardiovascular disorder. Therefore, the objective of the present study was to investigate the protective effect of concomitant administration of flax lignan concentrate and ω-3-FA on myocardial damage in doxorubicin-induced cardiotoxicity in Wistar rats.

2. Materials and methods

2.1. Collection and authentication of plant

Authenticated seeds of *Linum usitatissimum* (Linn.) were obtained from Dr. P.B. Ghorpade, Principal, Scientist, Punjabrao Deshmukh Krushi Vidyapeeth, College of Agriculture, Nagpur, India and voucher specimen was deposited at the institute. Flaxseeds were stored in cold room before processing. Further processing for oil extraction was carried out at our Omega-3-oil unit, Sangamner, Maharashtra, India.

2.2. Drugs and chemicals

ω-3-FA mainly containing docosahexaenoic acid derived from algal sources was obtained from Martek Biosciences Corporation, Columbia, USA. Doxorubicin (Dox) was received as a gift sample from Serum Institute of India, Pune, India. Absolute alcohol (Changshu Yangyuan Chemicals, China) was purchased from respective vendors. n-Hexane, hydrochloric acid, sodium hydroxide and sodium chloride of analytical grade were purchased from Qualigene fine-chem. Ltd., Mumbai, India. Rat tumor necrotic factor-α kit (Thermo Scientific, Rat TNF-α kit, Pierce Biotech Int., Rockford, IL, USA), was purchased from respective vendors. Triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL-C) kits ware purchased from Accurex Biomedical Pvt. Ltd., India. Epinephrine hydrochloride, super oxide dismutase (SOD) and malondialdehyde (MDA) were purchased from Sigma Chemical Co., USA. Reduced glutathione (GSH), 5,5′-dithiobis (2-nitro benzoic acid) (DTNB) and thiobarbituric acid (TBA) were obtained from Himedia, India.

2.3. Preparation of flax lignan concentrate (FLC)

Preparation of flax lignan concentrate was carried out as described previously [3]. Briefly, the flaxseed were subjected for oil extraction by double cold pressed technique which was carried out at Indian Council of Agricultural Research under National Agriculture Innovation Project, Omega-3-oil unit, Sangamner, Maharashtra, India. In order to remove more residual lipids from the matrix use of non-polar organic solvent such as hexane in a soxhlet apparatus was used, as they might interfere with the analysis of lignans. In order to liberate SDG from its polymeric lignan precursor by breaking the ester-linkages present in the complex, direct alkaline hydrolysis was preferred, hence defatted flaxseed cake was then hydrolyzed with 1 M aqueous sodium hydroxide for 1 h at room temperature with intermittent shaking, followed by extraction with 50% ethanol. In order to prevent the ionization of any functional groups in the aliphatic and aromatic part of the SDG molecule, the filtrate was acidified to pH 3 using 1 N hydrochloric acid. The filtrate was concentrated in rotary evaporator. An additional benefit of this process is the destruction of the cyanogenic glycosides yielding an extract free of cyanogenic glycosides. The yield of FLC was 14% (w/w). The powdered lignan rich extract was dissolved in distilled water to prepare the different