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Antihyperlipidemic activity of concomitant administration of methanolic fraction of flax lignan concentrate and omega-3-fatty acid in poloxamer-407 induced experimental hyperlipidemia

Anand A. Zanwar[a,b], Mahabaleshwar V. Hegde[b], Supada R. Rojatkar[c], Subhash L. Bodhankar[a,*]

[a] Department of Pharmacology, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Paud Road, Erandwane, Pune 411 038, Maharashtra, India

[b] Center for Innovation in Nutrition, Health, Disease, Interactive Research School for Health Affairs, Medical College Campus, Bharati Vidyapeeth Deemed University, Dhankawadi, Pune 411 043, India

[c] R & D Centre in Pharmaceutical Sciences and Applied Chemistry, Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandawane, Pune 411038, India

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ABSTRACT

Linum usitatissimum (Linn.), commonly known as flaxseed, which is a good source of dietary fiber and lignans. Secoisolariciresinol diglucoside (SDG) from flaxseed and omega-3 fatty acids (n-3-FA) from algal source have been shown to have multiple health benefits including cardiovascular disorders and related disorders. The objective of present study was to prepare methanolic fraction of flax lignan concentrate (MF-FLC) and to investigate the antihyperlipidemic activity of MF-FLC and omega-3-fatty acid alone and in combination after acute administration in poloxamer-407 induced hyperlipidemic rats. Solvent–solvent extraction method revealed around 3 times increase in SDG lignan content than that of flax lignan concentrate. Acute hyperlipidemia was induced in rats using poloxamer 407 (1 ml of 30%, w/v; i.p.). Concomitant administration of MF-FLC (100 mg/kg) and n-3-FA (1 ml/kg) significantly reduced serum cholesterol ($p < 0.001$), triglyceride ($p < 0.001$) and very low density lipoprotein ($p < 0.001$) whereas high density lipoprotein showed significant increase ($p < 0.05$). It can be concluded that supplementation of MF-FLC and n-3-FA can be useful for prevention/control of hyperlipidemia.

1. Introduction

The increase in deaths due to coronary heart disease has been blamed on the increased consumption of saturated fats. Every year about 715,000 Americans have a heart attack (Roger et al., 2012). Ischemic heart disease following atherosclerosis is a giant killer and the incidence of atherosclerosis in coronary arteries is rapidly increasing. The increased amount of atherosclerosis (advanced and intermediate lesions) found in the young population (Thej et al., 2012). Hypolipidemic pharmaceutical agents, such as atorvastatin, cholestyramine, colestipol, lovastatin, pravastatin and niacin are effective in various degrees in reducing serum triglycerides and low-density lipoprotein-cholesterol (LDL-C) and increasing high density lipoprotein-cholesterol (HDL-C). However, these drugs also have significant side effects. The statins may elevate liver enzymes, disturb the gastro intestinal tract. Many billions of dollars are spent annually on these drugs. Better nutrition with more long-chain omega-3 fatty acids, especially docosahexaenoic acid, can produce the same lipid changes and positive effects with no side effects and much less expense (Horrocks and Yeo, 1999). Cognition problems associated with statin therapy have variable onset and recovery courses, which suggest the need of alternative therapy (Golomb and Evans, 2008).

Linum usitatissimum (Linn.), commonly known as flaxseed or linseed belongs to the family Linaceae. Flaxseed exists in 4 main forms: whole seed, ground, partially defatted flaxseed meal, or flaxseed oil. Whole flaxseed contains about 35% oil of which 55% is alpha-linolenic acid. Flaxseed is also a good source of dietary

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapantaenoic acid; FLC, flax lignan concentrate; FDA, food and drug administration; HDL-C, high density lipoprotein-cholesterol; HPTLC, high performance thin layer chromatography; HDL-C, high-density lipoprotein cholesterol; i.p., intraperitoneal; LDL-C, low density lipoprotein-cholesterol; MF-FLC, methanolic fraction of flax lignan concentrate; n-3-FA, omega-3 fatty acids; P-407, poloxamer-407; PUFA, poly unsaturated fatty acid; SDG, secoisolariciresinol diglucoside; TLC, thin layer chromatography; TC, total cholesterol; TG, triglycerides; VLDL-C, very low-density lipoprotein-cholesterol.

* Corresponding author. Tel.: +91 20 25437237x29; fax: +91 20 25439386.
E-mail addresses: sbodh@yahoo.com, anandzanwar@rediffmail.com (S.L. Bodhankar).

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fiber and lignans (Bassett et al., 2009). The major lignan in flaxseed is secoisolariciresinol diglucoside (SDG) which is known for several pharmacological actions. Smaller quantities of other lignan such as matairesinol, isolariciresinol, lariciresinol, demethoxysecoisolariciresinol and pinoresinol have also been identified in flaxseed (Johnsson et al., 2000). Several research groups have reported the extraction of lignan from flaxseed; however, yield of SDG is very low. The microwave-assisted extraction of secoisolariciresinol diglucoside is found to have more yield (Nemes and Orsat, 2011). Extraction from natural raw materials and chemical synthesis is easy way to attain extraction and purification of active compound is very tedious process (Sainvitu et al., 2012). So there is need to have simple solvent extraction method which will be better yield.

The antiatherogenic activity of SDG lignan is reported (Prasad, 1997, 2005; Prasad et al., 1998). Recently, dietary flaxseed shown regression of atherosclerotic plaques (Francis et al., 2013). Also Penumathsa et al. (2007, 2008) reported the protective effect of SDG in hypercholesterolemic myocardial infraction in rats. The association between lignans and decreased risk of cardiovascular disease is reviewed by Peterson et al. (2010). Also (−)-secoisolariciresinol shown suppressive effect on the gain of body weight of mice by inducing gene expression of adiponectin in mice fed with high-fat diet (Tominaga et al., 2012).

Increased intakes of all three of the main omega-3 fatty acids (n-3-FA) i.e. alpha-linolenic acid, eicosapantaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to have multiple health benefits. The strongest evidence for cardiovascular benefit is related to increased intakes of EPA and DHA. The U.S. Food and Drug Administration (FDA) has permitted a qualified health claim for foods and dietary supplements containing EPA and DHA, saying: "supportive but not conclusive research shows that consumption of EPA and DHA omega-3-fatty acids may reduce the risk of coronary heart disease". So there is need of systematic study to further confirm the health claims of omega-3-fatty acids for cardiovascular disease and also in atherosclerosis (US FDA, 2004). Supplementation with DHA from an algal source at appropriate dosages has been found to have beneficial effects alleviating risk factors for coronary heart disease (Holub, 2009).

Available literature on omega-3-fatty acid is mainly focused on dietary supplementation studies. Nutrition is not pharmacology and nutritional data should be analyzed carefully before being extrapolated to pharmacologic applications (De-Lorgeril and Salen, 2002). Also many of the studies with respect to n-3-poly unsaturated fatty acid (PUFA) are diet based studies which are prone for its oxidation and can vary the results. Indicating need of systematic study to confirm the cardioprotective action of n-3-PUFA which should avoid these problems. In present study we have selected DHA (n-3-PUFA/n-3-FA) from algal source and oral administration of n-3-FA was preferred. Cardioprotective benefits of combined administration of n-3-PUFA and plant sterol supplementation in hyperlipidemic individuals is studied, wherein n-3-PUFA and plant sterols as lipid-lowering agents provided greater risk reduction compared to either of the supplements alone (Micallef and Garg, 2009). In view of the available literature, we hypothesized that flax lignan alone or in combination with n-3-FA may play role in prevention of hyperlipidemia. It was therefore thought worthwhile to use pharmacological approach to evaluate antihyperlipidemic action of flax lignan and omega-3-fatty acid alone and their co-administration by oral route in laboratory animals. The literature survey revealed that earlier reports are non-existent using this approach. Previously we have reported cardioprotective and antihyerlipidemic action of flax lignan concentrate (FLC) (Zanwar et al., 2011, 2012). Recently, we have reported additive effect of concomitant administration of FLC and n-3-FA in doxorubicin induced cardiotoxicity (Zanwar et al., 2013).

Hence the objective of present study was to carry out solvent–solvent fractionation of FLC to prepare methanolic fraction of flax lignan concentrate (MF-FLC) and to investigate the antihyperlipidemic activity of MF-FLC and omega-3-fatty acid alone and in combination after acute administration in poloxamer-407 induced hyperlipidemic rats.

2. Materials and methods

2.1. Collection and authentication of plant

Authenticated seeds of *Linum usitatissimum* (Linn.) (variety NL-97) 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 collected from Yeotmal district of Maharashtra state during 2011. Flaxseeds were stored in cold room before processing. Flaxseeds were processed for oil extraction at our Real World Nutrition Laboratory, under Indian Council of Agricultural Research for National Agriculture Innovation Project.

2.2. Drugs and chemicals

Omega 3 fatty acid (n-3-FA) mainly containing docosahexaenoic acid derived from algal sources was obtained from Martek Biosciences Corporation, Columbia, USA. Poloxamer-407 (P-407) was obtained from Sigma–Aldrich, USA. Absolute alcohol (Changshu Yangyuan Chemicals, China) were purchased from respective vendors. n-Hexane, hydrochloric acid, sodium hydroxide were purchased from Qualigene Fine-Chem. Ltd., Mumbai, India. Solvents (ethyl acetate, methanol, formic acid) and precoated silica gel plates of 0.2 mm thickness (silica gel 60 F-254) used for present study were procured from Merck Ltd. Mumbai, India. All the chemicals used were of analytical grade.

2.3. Preparation of methanolic fraction of flax lignan concentrate (MF-FLC)

Preparation of flax lignan concentrate was carried out as described previously (Zanwar et al., 2013). Briefly, flaxseed cake was defatted by n-hexane. Defatted cake was then hydrolyzed followed by extraction with alcohol. The filtrate was acidified. The filtrate was dried using rota vac. The dry powder of hydroalcoholic extract was labeled as flax lignan concentrate (FLC).

Further for purification solvent–solvent fractionation on FLC was carried using ethanol, methanol and water with different concentration at different temperature conditions, further resultant fraction (methanolic fraction of flax lignan concentrate-MF-FLC) at subsequent step was subjected for qualitative high performance thin layer chromatography (HPTLC) analysis for secoisolariciresinol diglucoside content. The yield of MF-FLC was 15% (w/w). The powdered MF-FLC was dissolved in distilled water to prepare the different concentrations of MF-FLC and used for pharmacological studies. Fresh drug solution was prepared for each day.

2.4. Preparation of standard and MF-FLC solutions for chromatographic analysis

Stock SDG standard solution (concentration 2 mg/ml) was prepared by dissolving 2 mg of pure SDG in 1 ml of double distilled water to prepare stock solution. This stock solution was diluted 10 times to prepare working solution. Further working solution was prepared by further dilution in methanol to make final dilution as 400, 800, 1200, 1600, 2000, 2400 and 2800 ng. MF-FLC was dissolved in distilled water and further appropriate dilutions were made in methanol. MF-FLC and standard SDG were applied on thin