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# Omega-3-fatty acid adds to the protective effect of flax lignan concentrate in pressure overload-induced myocardial hypertrophy in rats via modulation of oxidative stress and apoptosis

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ABSTRACT

Objective of the present investigation was to study the effect of the flax lignan concentrate (FLC) and Omega-3-fatty acid (O-3-FA) on myocardial apoptosis, left ventricular (LV) contractile dysfunction and electrocardiographic abnormalities in pressure overload-induced cardiac hypertrophy. The rats were divided into five groups such as sham, aortic stenosis (AS), AS + FLC, AS + O-3-FA and AS + FLC + O-3-FA. Cardiac hypertrophy was produced in rats by abdominal aortic constriction. The rats were treated with FLC (400 mg/kg, p.o.), O-3-FA (400 mg/kg, p.o.) and FLC + O-3-FA orally per day for four weeks. The LV function, myocardial apoptosis, and oxidative stress were quantified. FLC + O-3-FA treatment significantly reduced hemodynamic changes, improved LV contractile dysfunction, reduced cardiomyocyte apoptosis and cellular oxidative stress. Moreover, it significantly up-regulated the VEGF expression and decreased TNF-alpha level in serum. The histological analysis also revealed that FLC + O-3-FA treatment markedly preserved the cardiac structure and inhibited interstitial fibrosis. In conclusion, FLC + O-3-FA treatment improved LV dysfunction, inhibited cardiomyocyte apoptosis, improved myocardial angiogenesis, conserved activities of membrane-bound phosphatase enzymes and suppressed inflammation through reduced oxidative stress in an additive manner than FLC alone and O-3-FA alone treatment in pressure overload-induced cardiac hypertrophy.



## 1. Introduction

Cardiac hypertrophy is a hemodynamic response to elevated afterload that develops due to pressure or volume overload. It has been reported that gradual aortic constriction for four to eight weeks produces stable LV hypertrophy in rats [1]. Although LV hypertrophy is the most important compensation mechanism in chronic pressure overload, it has frequently been associated with heart failure and death [2,3]. It occurs because the hypertrophied myocardium presents structural changes that lead to cardiac remodeling, such as a disproportional increase in the myocyte component compared to vascular component [4]. One of the consequences is an imbalance between oxygen supply and consumption, which causes myocyte necrosis, apoptosis, and abnormal collagen accumulation [5,6].

Oxidative stress is known to play a critical role in inducing myocardial remodeling and hypertrophy in animal models of chronic pressure overload [7,8]. Myocardial cells can scavenge free radicals effectively by means of antioxidants. Free radical scavenging enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-S-transferase (GST) are the first line of cellular defense against oxidative injury [9,10]. These enzymes are lowered due to enhanced lipid peroxidation. Vascular endothelial growth factor (VEGF) is an important mediator of angiogenesis that promote blood vessel growth during embryonic development and tumorigenesis [11]. It has been reported that VEGF stimulated proliferation and migration of endothelial cells and played a pivotal role in vasculogenesis, angiogenesis, and endothelial integrity and survival [12].

Recent clinical and preclinical data suggest that a high intake of the Omega-3 polyunsaturated fatty acid (O-3-FA) containing eicosapentaenoic acid and docosahexaenoic acid from fish oil prevent the development and progression of cardiac hypertrophy and heart failure [13]. It is also reported in dietary guidelines that high intake of eicosapentaenoic acid and docosahexaenoic acid can reduce the risk for coronary heart disease [14]. Supplementation of O-3-FA derived from fish oil is found effective clinically in the treatment of hypertriglyceridemia and may reduce serious arrhythmias and sudden cardiac death [15]. Flaxseed is the richest source of plant lignans. Cardioprotective activity of flax lignan concentrate (FLC) has been studied earlier in our laboratory in the rat model of myocardial necrosis induced by isoprenaline [16]. It has been reported that n − 3 polyunsaturated α-linolenic acid (ALA) showed protective action

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in cardiac degenerative diseases via regulation of caveolin-3 and inhibition of TNF-α expression [17,18]. Another study carried out by researcher in hamster showed that ALA enriched diet prevents myocardial damage via modulation of atrial natriuretic peptide (ANP), transforming growth factor-β1 (TGF-β1) [19]. Secoisolariciresinol diglycoside (SDG) a flaxseed lignan has been shown to be effective in lowering myocardial apoptosis and improving the LV contractile function in rat heart of ischemia–reperfusion injury [20]. Penumathsa et al. have previously reported that SDG is an angiogenic and cardioprotective agent against myocardial ischemia–reperfusion injury, and this effect is mediated by vascular endothelial growth factor nitric oxide pathways [21]. The effects of O-3-FA in combination with SDG concentrated flaxseed extract in the cardiac function have not been reported. Compared with fish oil, little is known about the effects of SDG concentrated flaxseed extract. Hence, the objectives of the present investigation were to assess the effect of FLC and O-3-FA on LV contractile function, electrocardiographic abnormalities, myocardial apoptosis and to determine cardiac marker enzymes in the rat model of pressure overload-induced cardiac hypertrophy by aortic stenosis.

## 2. Materials and methods

### 2.1. Collection and authentication of plant

Authenticated seeds of *Linum usitatissimum* (variety NL-97) were obtained from Dr. P. B. Ghorpade, Principal, Scientist and linseed breeder, Punjabrao Deshmukh Krushi Vidyapeeth, College of Agriculture, Nagpur, Maharashtra State, India and voucher specimen was deposited at the Institute.

### 2.2. Preparation of flax lignan concentrate (FLC)

The authenticated seeds of *L. usitatissimum* (variety NL-97) were procured and processed for extraction of lignan by the methods as described previously [22,23]. Briefly, the seeds of *L. usitatissimum* were processed for extraction of lignan-concentrated oil at our omega-3-oil unit (Sangamner, Maharashtra, India; set up under National Agriculture Innovation Project funded by Indian council of Agricultural Research, New Delhi, India). The double cold-pressed flaxseed cake/meal obtained from this oil unit was de-fatted by n-hexane in a soxhlet apparatus to remove residual oil. The de-fatted cake was then hydrolysed with 1 m aqueous sodium hydroxide for 1 h at room temperature with intermittent shaking, followed by extraction with 50% ethanol. The filtrate was acidified to pH 3 using 1 m hydrochloric acid. The filtrate was dried on a tray dryer at 50 °C. The yield of dry powder was 14.81% w/w. The lignan (SDG) content in the flax lignan concentrate extracted from *L. usitatissimum* was 40 mg/g.

### 2.3. Pilot study of dose fixation of FLC and O-3-FA

FLC at the dose of 200 and 400 mg/kg/day and O-3-FA at the dose of 1 ml/kg and 2 ml/kg (i.e., 200 mg/kg and 400 mg/kg) were screened previously in pressure overload-induced cardiac hypertrophy in rats. The optimum dose exhibiting maximum cardioprotective effect during study period was evaluated by measuring cardiac marker enzymes such as creatine phosphokinase myocardial bodies (CKMB), lactate dehydrogenase (LDH), electrocardiographic abnormalities, assessment of LV contractile function and histopathological analysis. FLC (400 mg/kg) and O-3-FA (400 mg/kg) were found to be most effective in histopathological and biochemical recovery. Hence, these doses were selected for the investigation.

### 2.4. Drugs and chemicals

O-3-FA used in the present study was a commercial product containing docosahexaenoic acid (DHA; 22: 6n − 3) from Martek Biosciences, Corporation, Columbia, USA. It contains 400 mg DHA. CK-MB, LDH and AST kits were purchased from Accurex Biomedical Pvt. Ltd., Mumbai, India. Rat TNF-α ELISA kit was purchased from Thermo Scientific, Rockford, IL, USA. All chemicals used were of analytical grade. Total RNA Extraction Kit and One-step RT-PCR kit were purchased from MP Biomedicals India Private Limited, India.

### 2.5. Experimental animals and research protocol approval

Male Wistar rats (250–300 g) were purchased from National Toxicology Centre, Pune, India. Rats were housed in an air-conditioned room having temperature 22 ± 2 °C, relative humidity 45 to 55% and 12-h light:12-h dark cycle. The animals had free access to standard food pellets (Chakan Oil Mills, Pune, India), and water was available ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

### 2.6. Experimental design surgical procedure

The animals were anesthetized with sodium thiopental (35 mg/kg) intraperitoneally (i.p.). A mid-abdominal incision was made to expose the abdominal aorta. The aorta above the left renal artery was dissected and constricted at the suprarenal level using a cannula of size 0.9 into 40 mm, which was ligated with aorta and withdrawn afterward. In an age and body weight matched sham-operated rat, the abdominal aorta was isolated and placed without ligation. After surgery, the rats were administered with penicillin (200 kU/kg/d) intramuscularly (i.m.) for 1 week to prevent infection [24,25]. One week after surgery, the AS rats were randomly divided into following groups:

Group I: Sham group: Rats received saline for 4 weeks, p.o.
Group II: Aortic stenosis (AS) group: Rats received saline for 4 weeks, p.o.
Group III: AS + FLC group: Rats received FLC (400 mg/kg, p.o.) for 4 weeks.
Group IV: AS + O-3-FA group: Rats received O-3-FA (400 mg/kg, p.o.) for 4 weeks.
Group V: AS + FLC + O-3-FA group: Rats received FLC (400 mg/kg, p.o.) and O-3-FA (400 mg/kg, p.o.) for 4 weeks.

Animal care was taken in accordance with institutional guidelines. The animals were anesthetized with urethane injection (1.25 g/kg i.p.) on the last day of the treatment and subjected to the measurement of electrocardiographic abnormalities, hemodynamic changes, and LV contractile function. Blood samples were collected after hemodynamic recording and centrifuged at 7500 rpm for 15 min at 0 °C. Serum was isolated and transferred by using a micropipette in Eppendorf tubes and stored at 4 °C till analyzed.

### 2.7. Assessment of electrocardiographic abnormalities

For the measurement of the electrocardiogram, the leads were placed on the right foreleg (negative electrode), left foreleg (positive electrode) and right hind leg (neutral electrode). Electrocardiographic changes were recorded using 8 channels Power Lab System (AD Instruments Pty Ltd with LABCHART 6 software, Unit 13, 18-22 Lexington Drive, Bella Vista NSW 2153, Australia) [26,27].

### 2.8. Invasive measurement of hemodynamic changes and LV contractile function

Blood pressure was measured by means of a polyethylene cannula (PE 50) filled with heparinized saline (100 IU/ml) and connected to a pressure transducer. The cannula was connected to a transducer, and