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Research Article
Flax Lignan Concentrate Ameliorates Nω-Nitro-L-Arginine Methyl Ester (L-NAME)-Induced Hypertension: Role of Antioxidant, Angiotensin-Converting Enzyme and Nitric Oxide

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Abstract

Background: Recently the antihyperlipidemic, cardioprotective and *in vitro* antioxidant activity of Flax Lignan Concentrate (FLC) obtained from *Linum usitatissimum* Linn. (Linaceae) has been reported. The present study was aimed to assess the antihypertensive effect of FLC in L-NAME-induced hypertensive rats. **Materials and Methods:** Wistar rats (200-240 g) were given L-NAME (40 mg kg^{-1} b.wt. day^{-1}, p.o.) in drinking water for 4 weeks to induce hypertension. Rats were randomly divided into six groups: Control, L-NAME control, captopril (30 mg kg^{-1}, p.o.) and FLC (200, 400 and 800 mg kg^{-1}, p.o.). The FLC and standard drug captopril (30 mg kg^{-1}) were also administered daily for 4 weeks. **Result:** The FLC (400 and 800 mg kg^{-1}) significantly ($p<0.01$ and $p<0.001$) and dose-dependently decreased the elevated Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial Blood Pressure (MABP). It also normalized the altered left ventricular End Diastolic Pressure (EDP), dP/dt max. and dP/dt min. It also prevented the increase in heart weight and a decrease in heart rate and body weight. Undesirable changes, such as increased malondialdehyde (MDA) level and decreased the concentration of enzymatic antioxidants superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione (GSH) in the tissues (heart and aorta) were rectified after the administration of FLC (400 and 800 mg kg^{-1}). Oral administration of FLC (400 and 800 mg kg^{-1}) decreased Angiotensin Converting Enzyme (ACE) and also prevented the decrease in nitrite and nitrate concentration and cyclic guanosine monophosphate (cGMP) levels in plasma and tissues (heart and aorta). **Conclusion:** These finding suggested that the antihypertensive property of FLC in L-NAME hypertensive rats may be because of increased bioavailability of NO, ACE inhibition and antioxidant nature.

Key words: Flax lignan concentrate, antihypertensive activity, L-NAME hypertension, nitric oxide, angiotensin converting enzyme

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hypertension is a social health problem and the most important risk factor for the development of severe cardiac diseases like ischemic heart disease, stroke, renal failure and heart failure[1-3]. Although some advanced drugs have been developed in recent years to control blood pressure, the management of hypertension without any side effects and maximum patient adherence remains a challenge in front of the researchers[4-6]. Monotherapy on hypertension used today is having limited efficacy and undesirable effects. Therefore, research and development of the drugs having multiple therapeutic effects are most advantageous[7]. Research and development of therapeutic agents obtained from the natural sources, especially those from plant sources have been raising nowadays[8,9]. Many patients turn to traditional herbal medicine for the management of hypertension because the modern drug therapy is too expensive for them[10,11].

Therapeutically, plant lignan has been becoming more popular nowadays because of their recognized health beneficial effect such as antitumor, antioxidant and both estrogenic and antiestrogenic activity[12]. Flaxseed is the highest source of lignan with secoisolariciresinolc diglucoside (SDG) as a key compound (percentage of yield being 0.37%). The concentration of SDG in flaxseed is about 1000 times as high as found in other food sources[13]. Although, human studies are limited, its pharmacological actions explain its antiatherogenic[14,15], antioxidant, anticancer, antiviral, bactericidal and anti-inflammatory[16-20], antidiabetic[21], antihyperlipidemic[22], cardioprotective[23,24] and renoprotective[25,26] activity.

It is well known that Reactive Oxygen Species (ROS) is altered in L-NAME-treated animals. Importantly, several studies have been reported that ACE inhibitor and angiotensin II (Ang II) receptor blocker drugs prevent the elevation of blood pressure in L-NAME induced hypertension model[27,28]. Endothelium-derived relaxation factor Nitric Oxide (NO) plays an important role in the regulation of vascular tone[29]. In hypertension elevated level of the reactive oxygen species, reduced bioavailability of NO and altered endothelial Nitric Oxide Synthase (eNOS) activity are a common etiological factor considered for the endothelial dysfunction[30,31]. The NO stimulates guanylyl cyclase (sGC), which converts guanosine triphosphate to cyclic guanosine monophosphate and plays an important role in activation of cyclic guanosine monophosphate (cGMP) dependent protein kinase (PKG) and resultant vascular smooth muscle relaxation[32].

Some of the *in vivo* efficacy observed with secoisolariciresinol diglucoside (SDG); a main constituent of FLC in normal and angiotensin-I induced acute hypertensive rats on blood pressure could be due to its effects on guanylate cyclase-nitric oxide pathway and Angiotensin Converting Enzyme (ACE), respectively[33,34]. Recent clinical studies revealed that flaxseed used as diet in peripheral artery disease patients for the duration of more than three months reduced blood pressure[35,36]. Hence, the aim of the present study was to evaluate the effect of FLC on blood pressure, antioxidant activity, ACE inhibiting mechanism and bioavailability of nitric oxide in L-NAME-induced hypertensive rats.

MATERIALS AND METHODS

Experimental protocol: Male wistar rats weighing (200-240 g) were procured from National Toxicology Centre, Pune, India. They were housed at 25±1°C temperature and 45-55% relative humidity with a 12 h light/dark cycle. The animals received standard food pellets (manufactured by Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum*. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Poona College of Pharmacy, Bharati Vidyapeeth Deemed University, Erandawane, Pune. The IAEC was constituted as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSA), India. The IAEC approval number is CPCSEA/PCL/01/2015-16.

Drugs and chemicals: The N_{ω}-nitro-L-arginine methyl ester (L-NAME), captopril, sulfanilic acid and N-(1-naphthyl) ethylene diamines were purchased from Sigma-Aldrich Corporation, USA. Absolute alcohol (manufactured by Changshu Yangyuan Chemicals, China) was purchased from the respective vendor. The cGMP ELISA kit was purchased from Cayman Chemical Company (Ann Arbor MI (USA)). All other chemicals were purchased from Qualigenes Fine Chemicals Pvt. Ltd., Mumbai, India or Merck, Mumbai, India.

Collection and authentication of plant seeds: *Linum usitatissimum* (Flaxseeds) were obtained from Punjabrao Deshmukh Krushi Vidyapeeth, College of Agriculture and Nagpur, India. A voucher specimen was deposited at Poona College of Pharmacy, Pune, India. The flaxseeds were stored in a cold place before processing for oil extraction. Oil extraction was carried out at our Real World Nutrition Lab, Bharati Vidyapeeth Deemed University, Pune, India.

Preparation of flax lignan concentrate: Preparation of FLC was carried out as described previously[37]. The flaxseed cake was defatted by n-hexane to remove residual oil. The defatted cake was then hydrolyzed with 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 using rotavac apparatus at 50°C. The dry powder of hydroalcoholic extract was labeled as FLC.

Preparation of drug solution and selection of FLC dose: Captopril and FLC were dissolved in distilled water. This study was carried out using three doses of FLC (i.e., 200, 400 and 800 mg kg^{-1}, p.o.) and one dose of captopril (i.e., 30 mg kg^{-1}, p.o.).

Experimental design and protocol for L-NAME-induced hypertension: Hypertension was induced by giving L-NAME in drinking water at a concentration of 0.4 mg mL^{-1} to account for a daily intake of 40 mg kg^{-1} throughout the experimental period (4 weeks)[38]. The rats were randomly divided into six groups, each containing six rats as given below:

Group 1: Control (drinking water)
Group 2: L-NAME control (L-NAME 40 mg kg^{-1} day^{-1})
Group 3: L-NAME+captopril (30 mg kg^{-1} p.o.)
Group 4: L-NAME+FLC (200 mg kg^{-1} p.o.)
Group 5: L-NAME+FLC (400 mg kg^{-1} p.o.)
Group 6: L-NAME+FLC (800 mg kg^{-1} p.o.)

The FLC and captopril were dissolved in water and administered to the rats orally using an oral feeding needle once in a day (every morning) for four consecutive weeks. The L-NAME control rats received water as a vehicle. After the administration of the last dose, blood was collected in an anticoagulant containing tube from all the rats by retro-orbital puncture. The blood was centrifuge for plasma collection and store at -80°C until further analyzed. At the end, all the animals were sacrificed for histopathological evaluation.

Assessment of hemodynamic changes: Assessment of hemodynamic changes was carried out as described previously[37,39]. At the end of 4th week, individual rats were anesthetized (urethane 1.25 g kg^{-1} i.p.$^{-1}$). The trachea was cannulated to support respiration. The Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and Mean Arterial Blood Pressure (MABP) were measured by invasive technique. A polyethylene cannula (PE 50) filled with heparinized saline (100 IU mL^{-1}) was inserted into the right carotid artery. The cannula was linked to a transducer and the signal was amplified.

The left ventricular hemodynamic changes were measured using a Millar mikro-tip transducer catheter (Model SRP-320, Millar instrument, INC 320-7051, Houston, Texas 77023-5417), inserted into the left ventricle via the right carotid artery and connected to a bioamplifier[40,41]. Maximum first derivative of ventricular pressure (dP/dt max.), minimum first derivative of ventricular pressure (dP/dt min.) and left ventricular end-diastolic pressure (EDP) signals were obtained from primary signals (left ventricular systolic pressure and blood pressure) by means of Powerlab 8-channel data acquisition system (AD Instruments Pvt. Ltd., with Lab Chart 7.3 Prosoftware, Australia).

Estimation of endogenous antioxidant enzyme: At the end of the experimental period of four weeks, the rats were humanely euthanized. The heart and aorta were excised and slices into pieces. The portions of the heart and aorta tissues were individually homogenized in 10% ice cold tris-hydrochloride buffer (10 mmol L^{-1}, pH 7.4) using tissue homogenizer (Remi, India) and centrifuged at 7500 rpm for 15 min. at 0°C. The clear supernatant was collected after the centrifugation and used for biochemical and molecular estimations. The level of malondialdehyde (MDA) in the heart and aorta tissues were measured by the method previously described[40,42-44] and the values were expressed in nanomoles of MDA mg^{-1} of protein. Enzymatic antioxidants Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) concentration were estimated according to previously described method[40,45-48]. The SOD, GPx and the catalase activity were expressed as U mg^{-1} of protein. The glutathione (GSH) assay was performed according to the method previously describe previously[49-52]. The amount of reduced glutathione was expressed as µg mg^{-1} of protein.

Determination of NO: The NO is highly unstable free radical, which is converted into stable metabolites nitrate and nitrite in the equimolar ratio[53-56]. The plasma and aortic tissue NO levels were determined as nitrite by the acidic Griess reaction. The principle of this assay is a reduction of nitrate

by vanadium. The nitrite reacts with sulfonamide and n-1-naphthyl ethylenediamine to produce a pink azo-product with maximum absorbance at 543 nm. The concentrations were calculated using a standard curve of sodium nitrate and the results were expressed in μmol L^{-1}.

Measurement of Angiotensin Converting Enzyme (ACE) and cyclic guanosine monophosphate (cGMP) activity: The ACE activity in heart and aorta was measured as described previously[57]. The heart and aorta were minced manually and homogenized in assay buffer (100 mM potassium phosphate, pH 7.4) using tissue homogenizer (Remi, India) and the homogenate was filtered through Whatman paper and the filtrate was centrifuged at 7500 rpm for 15 min. The supernatant was then used for subsequent analysis. The amount of cleaved hippuric acid from hippuryl histidyl-leucine (HHL) was measured by spectrophotometric method. Briefly, 50 μL of sample was added to 150 μL of HHL (8.33 mM) in 125 mM tris-HCL buffer (pH 8.3). Test and control tubes were incubated at 37°C for 30 min. After incubation, the enzymic reactions were terminated by addition of 250 μL of 1 M HCl. The hippuric acid produced by the reaction of the ACE on HHL is extracted into 1.5 mL of ethyl acetate from acidified solution. After centrifugation, 1 mL ethyl acetate layer was transferred to a clean tube and evaporated at 120°C for 30 min. Hippuric acid residue was then re-dissolved in 1 mL distilled water and the amount formed was determined from its absorbance at 228 nm. The ACE was expressed as mmol $min.^{-1}$ mg^{-1} protein. The GMP levels in the heart and aorta tissue homogenate were measured by using cGMP EIA kit obtained from Cayman Chemical Company, USA. The cGMP level was expressed as pmol mg^{-1} protein.

Histopathological examination: The heart and aorta tissue obtained from all experimental groups were cleaned and immediately fixed in a neutral buffered solution of 10% formalin[58]. The specimens were routinely processed and embedded in paraffin. The specimens were cut into sections of 5 μm thickness by microtome and stained with hematoxylin and eosin for microscopic examination. The sections were observed under the microscope and photomicrographs of the tissue section were taken using a microscope camera (Nikon Cool pix).

Statistical analysis: The data were expressed as Mean±Standard Error Means (SEM). Analysis of the data was performed using Graph Pad Prism 5.0 software (Graph Pad Software, Inc., USA). Hemodynamic and biochemical parameters were analyzed using one-way ANOVA and Dunnett's test was applied for *post hoc* analysis. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of FLC on blood pressure and left ventricular contractile function of heart: Figure 1 shows the effect of FLC on hemodynamic parameters at three different concentrations (200, 400 and 800 mg kg^{-1}) in I to VI groups after 4 weeks. The L-NAME control rats showed significant ($p<0.001$ each) increase in SBP (160.7±3.879 mmHg), DBP (123.9±2.891 mmHg), MABP (136.2±2.46 mmHg), EDP (14.28±0.95 mmHg), dP/dt max. (4249±126.6 mmHg sec^{-1}) and dP/dt min. (-4580±82.4 mmHg sec^{-1}) after 4 weeks. The FLC (800 mg kg^{-1}) treatment showed a significant ($p<0.001$) decrease in the SBP (128.3±2.57 mmHg), DBP (86.83±2.91 mmHg), MABP (100.6±1.78 mmHg), EDP (9.17±0.57 mmHg), dP/dt max. (3378±85.98 mmHg sec^{-1}) and dP/dt min. (-3706±102.9 mmHg sec^{-1}). The FLC (400 mg kg^{-1}) treatment also showed significant ($p<0.01$ each) decrease in SBP (140.2±4.57 mmHg), DBP (108.9±2.42 mmHg), MABP (124.2±2.82 mmHg), dP/dt max. (3614±115.8 mmHg sec^{-1}) and dP/dt min. (-4033±66.43 mmHg sec^{-1}) compared to L-NAME control animals. However, FLC in low dose (200 mg kg^{-1}) did not show any significant effect on hemodynamic parameters.

Effect of FLC on heart weight, body weight and heart rate: The L-NAME hypertensive rats significantly ($p<0.001$) decreased body weight and heart rate and increased heart weight. The treatments with captopril (30 mg kg^{-1}) and FLC (400 and 800 mg kg^{-1}) showed significant results by bringing back those values to near normal levels (Table 1).

Effect of FLC on lipid peroxides, enzymatic and non-enzymatic antioxidant enzymes: The MDA level in the tissues (heart and aorta) of L-NAME hypertensive rats elevated significantly ($p<0.001$). Oral administration of captopril (30 mg kg^{-1}) and FLC (800 mg kg^{-1}) significantly ($p<0.001$) decreased the levels of MDA in the tissues (heart and aorta) as compared to the treatment with FLC (400 mg kg^{-1}) ($p<0.01$ and $p<0.05$, respectively).

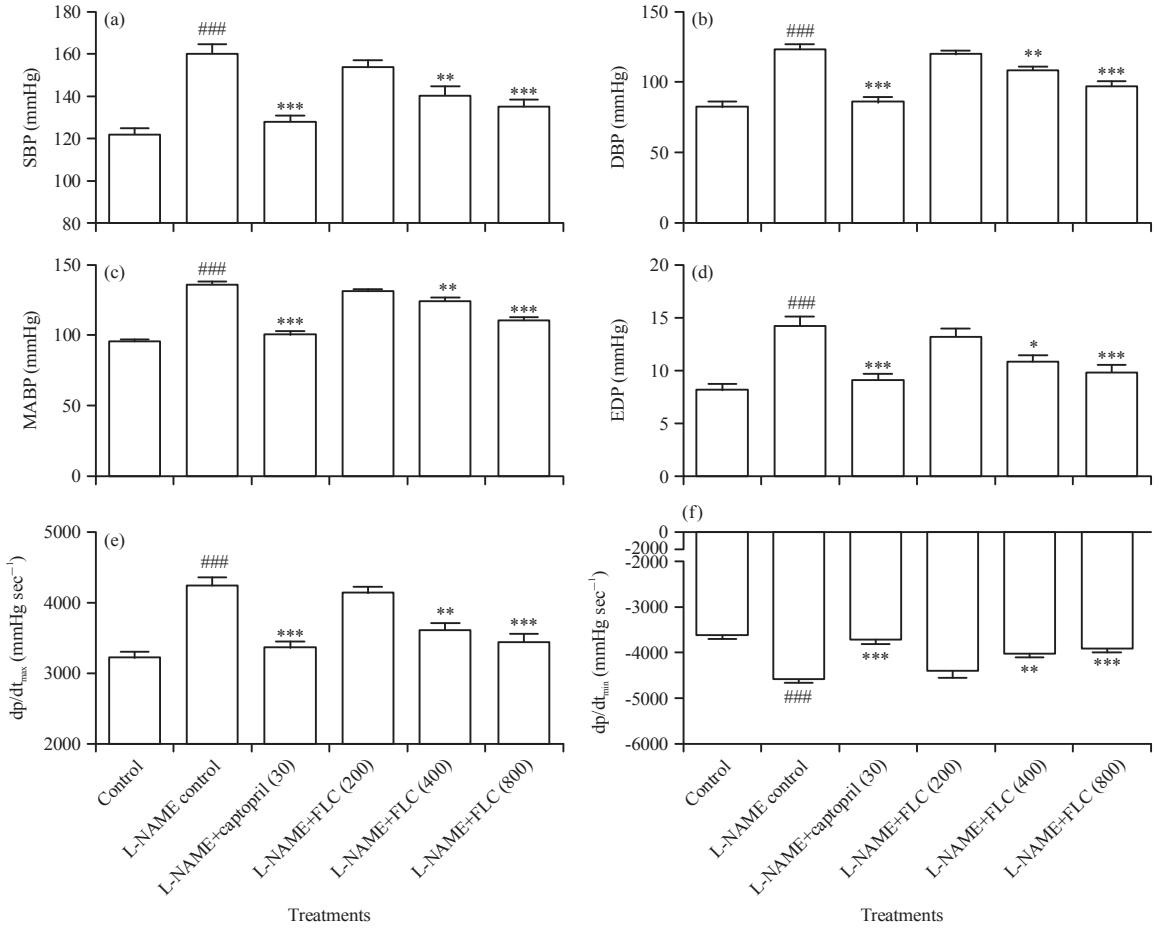

Fig. 1(a-f): Effect of FLC and captopril on (a) SBP, (b) DBP, (c) MABP, (d) EDP, (e) $dp/dt_{max.}$ and (f) $dp/dt_{min.}$ of heart, values are expressed as Mean±SEM for n = 6 rats. Data were analyzed by one-way ANOVA followed by Dunnett's test, ns: Non-significant, ###p<0.001 as compared with control group at same time point, whereas *p<0.05, **p<0.01 and ***p<0.001 as compared with L-NAME control group at same time point

Table 1: Effect of FLC and captopril on organs weight, body weight and heart rate in L-NAME hypertensive rats

	Treatment					
Parameter	Control	L-NAME control	L-NAME+captopril (30 mg kg^{-1})	L-NAME+FLC (200 mg kg^{-1})	L-NAME+FLC (400 mg kg^{-1})	L-NAME+FLC (800 mg kg^{-1})
Organ weight						
Heart (g)	0.86±0.05	1.20±0.031$^{###}$	0.881±0.051***	1.228±0.051^{ns}	0.974±0.031**	0.913±0.034***
Body weight (g)						
0th week	223.6±3.37	227.00±4.12^{ns}	226.50±5.27	226.50±4.39^{ns}	224.33±4.81^{ns}	223.00±3.91^{ns}
1st week	231.5±4.08	221.67±4.30^{ns}	233.50±6.02	227.33±3.96	230.50±4.70^{ns}	226.83±5.74^{ns}
2nd week	239.8±4.94	215.17±4.53$^{##}$	240.67±6.02**	231.00±3.55^{ns}	233.33±5.76*	234.67±5.76**
3rd week	248.5±5.12	214.83±4.00$^{###}$	246.83±5.44***	232.67±4.39^{ns}	239.00±5.86**	241.33±5.33***
4th week	255.3±4.56	205.33±4.36$^{###}$	251.83±5.34***	229.17±3.63**	244.83±6.11***	249.17±5.29***
Heart rate (beats min.$^{-1}$)						
0th week	364.50±4.62	355.33±4.51^{ns}	362.17±6.26^{ns}	364.00±2.56^{ns}	361.17±3.48^{ns}	363.00±5.03^{ns}
1st week	357.00±3.69	347.00±2.97^{ns}	351.83±2.60^{ns}	353.67±3.45^{ns}	351.83±2.60^{ns}	355.33±4.06^{ns}
2nd week	360.33±4.10	336.17±2.55$^{###}$	354.17±3.00**	344.50±3.17^{ns}	352.50±2.81*	355.67±3.90**
3rd week	362.67±4.63	340.33±3.84$^{###}$	358.00±2.50**	342.67±5.09^{ns}	355.33±3.01*	357.83±3.18**
4th week	359.17±3.32	327.00±4.04$^{###}$	359.33±4.13***	338.67±3.71^{ns}	345.83±2.80**	358.50±4.72***

Data was analyzed by Two-way ANOVA followed by *post hoc* Bonferroni tests, ns: Non-significant, ###p<0.001 as compared with control group at same time point, whereas *p<0.05, **p<0.01 and ***p<0.001 as compared with L-NAME control group at same time point

Table 2: Effect of FLC and captopril on endogenous antioxidant enzymes in L-NAME hypertensive rats

	Treatment					
Parameters	Control	L-NAME control	L-NAME+captopril (30 mg kg^{-1})	L-NAME+FLC (200 mg kg^{-1})	L-NAME+FLC (400 mg kg^{-1})	L-NAME+FLC (800 mg kg^{-1})
SOD (U mg^{-1} protein)						
Heart	5.25±0.22	3.39±0.14$^{\#\#\#}$	4.73±0.19***	3.52±0.16^{ns}	4.24±0.11**	4.58±0.17***
Aorta	5.12±0.18	2.84±0.13$^{\#\#\#}$	4.66±0.19***	3.07±0.17^{ns}	3.73±0.21**	4.43±0.17***
MDA (nmol mg^{-1} protein)						
Heart	4.88±0.19	9.02±0.27$^{\#\#\#}$	5.66±0.29***	8.01±0.40^{ns}	7.63±0.29**	6.66±0.29***
Aorta	0.91±0.05	1.38±0.11$^{\#\#\#}$	0.94±0.04***	1.30±0.05^{ns}	1.10±0.06*	0.99±0.04***
GPx (U mg^{-1} protein)						
Heart	7.77±0.24	3.65±0.33$^{\#\#\#}$	6.57±0.30***	3.88±0.25^{ns}	5.05±0.30**	5.34±0.20***
Aorta	6.02±0.39	3.90±0.16$^{\#\#\#}$	6.42±0.29***	4.04±0.22^{ns}	5.07±0.34*	6.26±0.22***
Catalase (U mg^{-1} protein)						
Heart	50.23±4.27	23.10±1.95$^{\#\#\#}$	42.73±2.66***	31.45±3.04^{ns}	39.93±3.21**	41.77±2.61***
Aorta	45.28±4.85	20.40±2.21$^{\#\#\#}$	42.64±2.22***	30.57±2.91^{ns}	35.89±2.55**	39.54±2.64***
GSH (μg mg^{-1} protein)						
Heart	8.74±0.20	3.69±0.25$^{\#\#\#}$	6.57±0.21***	3.98±0.30^{ns}	4.92±0.19**	5.98±0.25***
Aorta	12.03±0.38	4.58±0.35$^{\#\#\#}$	11.03±0.37***	5.01±0.18^{ns}	6.43±0.39**	9.05±0.36***

Values are expressed as Mean±SEM for n = 6 rats, data were analyzed by one-way ANOVA followed by Dunnett's test, ns: Non-significant, $^{\#\#\#}$p<0.001 as compared with control group at same time point whereas *p<0.05, **p<0.01 and ***p<0.001 as compared with L-NAME control group at same time point

However, FLC (200 mg kg^{-1}) treated group did not show any significant restoration (Table 2).

The activities of SOD, catalase and GPx in the tissues (heart and aorta) of L-NAME hypertensive rats were decreased significantly (p<0.001) after 4 weeks. Treatment with captopril (30 mg kg^{-1}) and FLC (800 mg kg^{-1}) significantly (p<0.001) restored the activities of SOD, catalase and GPx in the tissues (heart and aorta). The FLC (400 mg kg^{-1}) also showed significant (p<0.01) restoration of the SOD, catalase and GPx activity (Table 2).

The level of reduced glutathione (GSH) in the tissue (heart and aorta) was decreased significantly (p<0.001) in L-NAME-induced hypertensive rats. Captopril (30 mg kg^{-1}) and FLC (800 mg kg^{-1}) treatment significantly (p<0.001) improved values toward the normal. The FLC (400 mg kg^{-1}) group rats also showed significantly (p<0.01) restoration of GSH activity in the heart and aorta tissue, whereas FLC (200 mg kg^{-1}) treated group did not show any significant change in activity (Table 2).

Effect of FLC on nitrite/nitrate production: The L-NAME hypertensive rats showed significant (p<0.001) decrease in level of nitric oxide metabolite (nitrite/nitrate) in plasma (54.81±2.05 μmol L^{-1} vs. 84.54±5.77 μmol L^{-1}) and aortic tissue (66.03±5.07 μmol L^{-1} vs. 112.5±5.05 μmol L^{-1}). The rats treated with captopril (30 mg kg^{-1}) and FLC (400 and 800 mg kg^{-1}) showed significantly (p<0.001, p<0.01 and p<0.001, respectively) elevated plasma NO (81.07±3.35, 75.15±3.96 and 80.3±2.79 μmol L^{-1}, respectively) and aortic tissue NO (105.5±4.17, 87.97±3.3 and 97.38±3.34 μmol L^{-1}, respectively) level. However, FLC (200 mg kg^{-1}) did not show any significant effect (Fig. 2a, b).

Effect of FLC on Angiotensin Converting Enzyme (ACE) activity: The result as shown in Fig. 2c-d revealed that increased blood pressure in the L-NAME hypertensive rats was linked with significant (p<0.001) increase in the heart and aortic tissue ACE activity compared with control group. Captopril (30 mg kg^{-1}) and FLC (400 and 800 mg kg^{-1}) treatment showed the significant inhibitory effect on ACE activity in the heart and aortic tissue (Fig. 2).

Effect of FLC on cyclic guanosine monophosphate (cGMP) level: The level of cGMP in cardiac tissue and aortic tissue decreased significantly (p<0.001) in L-NAME hypertensive rats compared with control group. The treatment with captopril (30 mg kg^{-1}) and FLC (400 and 800 mg kg^{-1}) significantly (p<0.001, p<0.05 and p<0.01) elevated the cardiac and aortic tissue cGMP (Fig. 2). This effect was more prominent in FLC (800 mg kg^{-1}) treated rats.

Effect of FLC on histopathology of heart: The untreated control rat showed normal histopathological manifestation without any myocardial damage at the microscopic level in the heart. The L-NAME hypertensive group rats were found to have severe myocardial degeneration, hypertrophy and

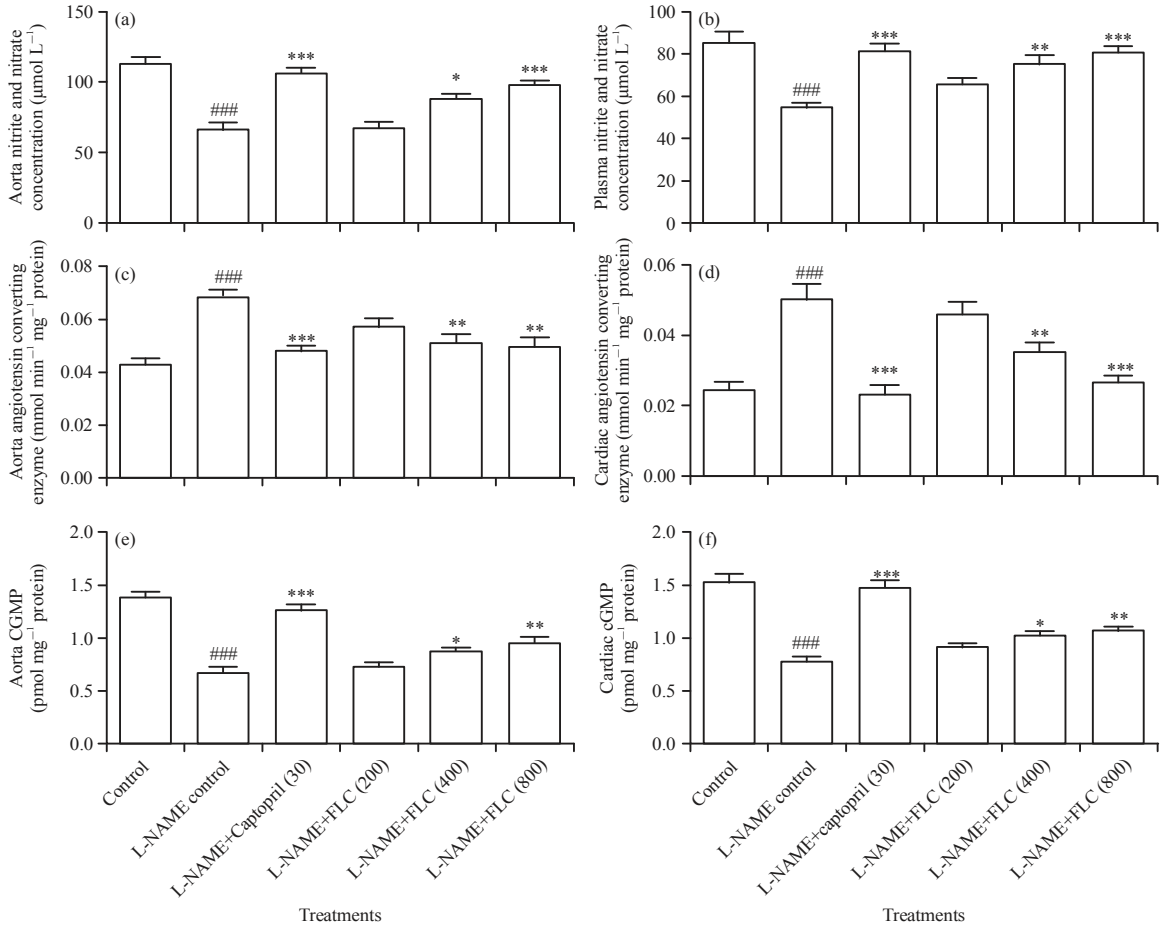

Fig. 2(a-f): Effect of FLC and captopril on nitrite/nitrate, angiotensin converting enzyme and cyclic guanosine monophosphate (cGMP) level in L-NAME hypertensive rats, values are expressed as Mean±SEM for n = 6 rats, data were analyzed by one-way ANOVA followed by Dunnett's test, ns: Non-significant, ###p<0.001 as compared with control group at same time point whereas *p<0.05, **p<0.01, ***p<0.001 as compared with L-NAME control group at same time point

fibrosis. Administration of captopril (30 mg kg^{-1}) and FLC (400 and 800 mg kg^{-1}) reduced this myocardial damage, collagen deposition and fibrosis. However, FLC (200 mg kg^{-1}) did not show any significant protection against L-NAME induced hypertension (Fig. 3a-f).

DISCUSSION

It is well known that the chronic inhibition of NO biosynthesis by the oral administration of Nitric Oxide Synthase (NOS) inhibitor L-NAME will generate hypertension, cardiac remodeling and vasoconstriction in rats[59,60]. Previous studies in the laboratory have reported that FLC contains SDG as the main constituent with the several other compounds like matairesinol, lariciresinol, hinokinin, arctigenin, pinoresinol and demethoxy secoisolariciresinol[37,61]. The SDG possess blood pressure lowering and ACE inhibitor like activity in the normal and hypertensive rat[15,62]. In this study, L-NAME control (hypertensive) rats showed a significant increase in SBP, DBP and MABP. However, treatment with FLC (400 and 800 mg kg^{-1}) and positive control drug captopril caused a significant reduction in SBP, DBP and MABP in the hypertensive rats. It is in agreement with the previous study, where the antihypertensive effect of FLC in DOCA-salt hypertensive rats was reported[61]. The current study also revealed that FLC treatment restored the altered left ventricular parameters (EDP, dP/dt max. and dP/dt min.) and offered sufficient cardiac contractile reserve in L-NAME hypertension.

Chronic hypertension is always associated with marked body weight loss in laboratory animals[63]. In the present investigation, it was found that L-NAME hypertensive group

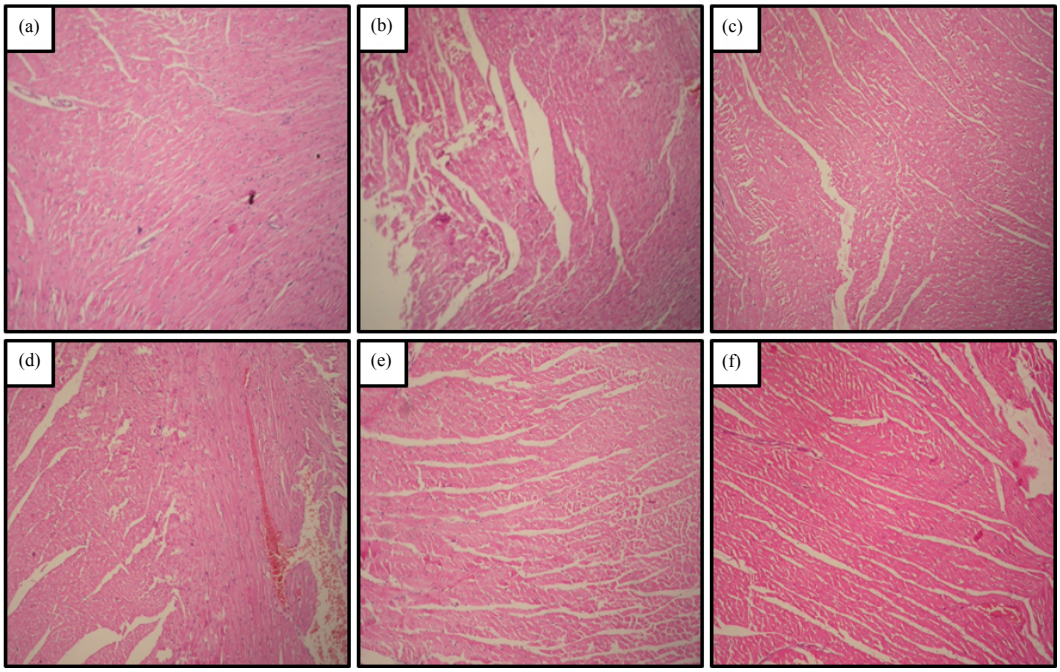

Fig. 3(a-f): Effect of FLC and captopril on histopathology of heart from (a) Normal (Control), (b) Untreated control (L-NAME control), (c) Captopril (L-NAME+captopril, 30 mg kg^{-1}), (d) FLC (L-NAME+FLC, 200 mg kg^{-1}), (e) FLC (L-NAME+FLC, 400 mg kg^{-1}) and (f) FLC (L-NAME+FLC, 800 mg kg^{-1}) treated rats

rat showed significantly loss of body weight. Treatment with FLC significantly improved the weight loss, which may be due to its ability to oppose hypertension and destruction of structural proteins. The L-NAME induced hypertension caused a sustained decrease in the heart rate in the study, in accordance with other reports[16,64,65]. The current results showed that treatment with the FLC significantly prevented the decrease in heart rate, which may be due to the antihypertensive effect of FLC in L-NAME hypertensive rats. The present study revealed that wet weight of heart was significantly increased in the L-NAME hypertensive group, which is in line with the previous studies. The treatment with the captopril and FLC prevented hypertrophy of heart and reduced the heart weight which may be due to their blood pressure lowering effect.

One of the possible mechanisms to explain the antihypertensive activity of FLC in L-NAME hypertensive rats is its pronounced antioxidant nature[37,61]. Oxidative stress in hypertension is an imbalance between the formation of Reactive Oxygen Species (ROS) and antioxidant defense mechanism. The present study demonstrated a marked elevation in lipid peroxidation measured as MDA concentration in the cardiac and aortic tissue represent significant damage due to high blood pressure, which in agreement with the previous study[66]. The FLC significantly protected the heart and aorta tissue from hypertension-induced oxidative damage as evidenced by the decreased level of MDA in L-NAME hypertensive rats may be due to its ability in decreasing ROS. Enzymatic antioxidants (SOD, catalase and GPx) and non-enzymatic antioxidant (GSH) protect cells in the body from the various oxygen radicals[67-71]. Enzyme SOD converts superoxide radical into hydrogen peroxide and catalase (or other enzyme glutathione peroxide) converts the hydrogen peroxide to oxygen and water[72-77]. The combination of SOD, catalase and GPx remove oxygen radicals and any alteration in physiological level may cause oxidative damage[78-80]. Results of the present study demonstrated that L-NAME hypertensive group rat showed significantly decreased level of SOD, catalase and GPx in the cardiac and aortic tissue. The FLC significantly improved the activity of enzymatic antioxidant in L-NAME-induced hypertensive rats. The GSH is playing an important role in scavenging residual free radicals escaping from decomposition by the antioxidant enzyme[81-83]. In agreement with the previous reports, L-NAME-induced hypertensive rats showed significantly decreased level of GSH[38]. Administration of FLC significantly elevated the level of GSH in this study, which suggested that FLC might be useful in

counteracting free radical induced oxidative stress caused by L-NAME induced hypertension in rats.

The ACE inhibition may be another possible mechanism that has been proposed to explain the blood pressure lowering activity of FLC in L-NAME hypertensive rats. There is the connecting link between NO synthesis and tissue ACE activity in L-NAME induced hypertension[84]. In this study, ACE activity was found to be elevated in both heart and aorta tissue, thereby contributing the increased blood pressure. In consistent with previous reports, L-NAME hypertensive rats showed significantly elevated activity of ACE in both heart and aorta tissue[84,85]. This study revealed that FLC treatment caused a significant decrease in heart and aorta tissue ACE activity. Inhibition of ACE activity in tissue could be linked to the collaborative effect of SDG, matairesinol, lariciresinol, hinokinin, arctigenin, pinoresinol and demethoxy secoisolariciresinol and various other phenolic compounds. The SDG a main lignan constituent of FLC has been reported to inhibit ACE in angiotensin-I induced acute hypertensive rats[33,34].

The L-NAME hypertension produced excessive reactive oxygen species, which further reacted with NO and reduced its bioavailability[86]. Peroxynitrite and related compounds produced due to the oxidation of NO further induced oxidative stress and hypertension[87-91]. Plasma and aortic tissue nitrite/nitrate levels were significantly improved in this study revealed the ability of FLC to increase the bioavailability of NO by protecting it from free radical and thereby inhibiting its oxidation to peroxynitrite. The increase in plasma NO levels stimulate guanylate cyclase to increase the level of cGMP, which further causes vasorelaxation in arteries[92]. In the present study, cGMP levels in cardiac and aorta tissue decreased significantly in L-NAME hypertensive rats similar to the previous reports. Treatment with FLC reverses the reduction of cGMP in cardiac and aorta tissue explains its antihypertensive effect in L-NAME hypertensive rats.

CONCLUSION

In conclusion, results showed that FLC decreased blood pressure in L-NAME-induced hypertensive rats. Antihypertensive effects of FLC in L-NAME hypertensive rats were dose-dependent and at the highest dose (800 mg kg^{-1}) similar to those of captopril (30 mg kg^{-1}). In addition, FLC inhibited alterations in left ventricular function, oxidative stress and histopathological changes. Moreover, FLC showed promising results in normalizing the altered ACE activity, NO levels, cGMP levels and induced by L-NAME. These activities could propose a possible mechanism of action of FLC in this study. However, the observed effect could be attributed to the SDG and other phenolic compounds acting either synergistically or additively.

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