Favorable Effects of Flaxseed Supplemented Diet on Liver and Kidney Functions in Hypertensive Wistar Rats

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Abstract: Hypertension is a major risk factor for cardiovascular diseases and is detrimental to several organs including the liver and kidneys. The flaxseed-derived polyunsaturated fatty acids including the omega-3 and omega-6 essential fatty acids have been shown to blunt the effects of hypertension. It is however, unclear whether the flaxseed, which is rich in these essential fatty acids, could improve the liver and kidney dysfunctions observed in the hypertensive condition. To test this, functional markers of the liver and kidneys, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), uric acid (UA), creatinine, and renin were examined in hypertensive male Wistar rats fed a flaxseed diet. Normotensive rats maintained on a standard diet were rendered hypertensive with a daily administration of cyclosporin A (CYS) (25 mg/kg) for 4 weeks. Subsequently, hypertensive rats were either fed a standard diet alone or a flaxseed-supplemented standard diet (FLX; 10% W/W) for 8 weeks. Compared to normotensive rats, standard diet-fed hypertensive rats had significantly elevated blood pressure, altered lipid profile, and increased plasma levels of tissue markers measured immediately following the CYS treatment and thereafter at 4 and 8 week intervals. On the other hand, rats fed the FLX-supplemented diet had significantly lower blood pressure, an improved lipid profile and decreased tissue marker levels measured after 4 and 8 week durations. The data demonstrate for the first time the favourable effects of FLX in improving liver and kidney functions in the hypertensive condition. These effects are likely to be mediated by the alpha-linolenic acid (ALA) and linoleic acid (LA) contents of flaxseed oil due to its demonstrated ability to lower the blood pressure.

Key words: Flaxseed, hypertension, liver, kidney

1 INTRODUCTION

Dietary intervention using plant based products, has long been considered a better strategy for either preventing or reducing the progression of chronic diseases. This is largely due to the affordability of these products along with fewer side effects they produce compared to the drugs administered under a pharmacological approach¹⁾. The flaxseed, due to its diverse active components, is known to possess multiple health benefits against cardiovascular diseases, dyslipidemia, diabetes, obesity, and cancer²⁻⁵⁾. Flax (*Linum usitatissimum*), a nutritionally important oil seed plant, belongs to the Linaceae family. Flaxseed is an excellent source of oil, protein and dietary fibre as well as the phytochemical antioxidant, lignan (secoisolariciresinol diglucoside)^{6, 7)}. Flaxseed oil is rich in ALA, an omega-3 fatty acid and LA, an omega-6 fatty acid^{7, 8)}.

Hypertension is a chronic medical condition characterized by sustained elevated blood pressure⁹⁾. Several studies have indicated that a deficiency of ALA leads to the development of hypertension and the supplementation of flaxseed oil reverses the process and normalizes blood pressure^{7, 10-12)}. Hypertension is known to be detrimental to multiple organs including the liver and kidneys. Therefore, the present study was undertaken to examine whether flaxseed, with its major essential fatty acid content, could alleviate hypertension-induced liver and kidney dysfunctions and restore the functional markers of these organs. Therefore, we measured the effects of the flaxseed-supplemented diet on blood pressure, liver markers including aspartate AST and ALT. The kidney markers included BUN, UA, creatinine, and renin. These were all investigated in hypertensive male Wistar rats and compared to normoten-

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sive rats fed a standard diet.

2 EXPERIMENTAL

2.1 Animals

This study was conducted in accordance with the guidelines set by the ethical committee of King Abdulaziz University, Jeddah, Saudi Arabia. Male Wistar albino rats, 8 weeks old and 150-170 g in weight were acclimated to the environmental conditions of the animal housing facility for two weeks under a 12 h light-dark cycle and at $22 \pm 3^\circ\text{C}$ temperature. Rats were provided with water and a normal standard diet *ad libitum*.

2.2 Experimental diets

Compositions of the standard diet and the flaxseed-supplemented standard diet are presented in **Table 1**. Freshly ground flaxseed was obtained locally, roasted to remove cyanides, crushed and mixed with a standard chow diet at 10% (W/W) concentration. The diet was then moistened, re-pelleted, and fan-dried. Diets were refrigerated and protected from light. The flaxseed concentration used in this study (10 g/100 g) is similar to that used in studies reporting health-related benefits. Composition of the flaxseed supplemented diet (10% W/W) was calculated based on a 90:10 proportion of a standard diet and ground flaxseed respectively¹³⁾. The quantity of food consumed per day by each group of rats was calculated by subtracting the weight of the leftover chow from the weight of the initial chow provided. Total energy consumed per day was calculated based on the energy content of each diet and the amount of each diet consumed.

2.3 Experimental design

Rats were randomly assigned to three groups designated as control (normotensive, standard diet fed), CYS (hypertensive, standard diet fed) and CYS + FLX (hypertensive, flaxseed-supplemented standard diet fed) groups. Each

group contained 10 rats. Hypertension in the CYS group was induced by a daily subcutaneous administration of CYS (25 mg/kg diluted in 1 ml/kg olive oil) administered over a four week period. The CYS is an effective agent for the induction of hypertension and the CYS induced hypertensive rat models have been shown to exhibit the typical hypertensive characteristics^{14, 15)}. The mechanism of hypertension induction by CYS involves the immunosuppression mediated by the calcineurin inhibition, which is suggested to block either the T-cell signaling or the renal afferent activation^{16, 17)}. Normotensive rats received sham treatment. Hypertensive status was confirmed by measuring the blood pressure by the tail-cuff method. Rats in the control, CYS and CYS + FLX groups were initially maintained on a standard diet. This diet was replaced with flaxseed-supplemented diet for rats in the CYS + FLX group from the 29th day following the four week (28 days) cyclosporine A treatment. Diet supplementation was continued for eight weeks. Blood pressure was measured by the tail-cuff method and blood samples were collected by a retro-orbital bleeding at the beginning of the diet supplementation (baseline) and thereafter at 4- and 8- week intervals. Blood pressure was measured prior to the blood collection to avoid an influence of the blood collection procedure on the blood pressure. Plasma was separated from the blood by gradient centrifugation using Ficoll-Paque PLUS (GE Health Care, Germany) and stored at -80°C until analyzed.

2.4 Biochemical measurements

Plasma total-cholesterol and triacylglycerol (TAG) were measured using a fully automated analyzer (Konelab Instruments, Espoo, Finland). HDL-cholesterol levels were determined by phosphotungstic acid/magnesium chloride precipitation (Kone Instruments, Espoo, Finland). LDL-cholesterol was calculated using Friedewald equation. Plasma UA and creatinine were measured using an assay kit by following the manufacturer's instructions (BioAssay Systems, CA, USA). The BUN, was assayed by using a commercially available kit (Arbor Assays MI, USA), and renin activity was determined by using a renin assay kit (R&D Systems, MN, USA). Plasma AST and ALT activities were measured by following the manufacturer's instructions on their kit (Cayman Chemical Company, MI, USA).

2.5 Statistical analysis

SPSS statistical software was utilized for the data analysis. Data is represented by mean $\pm$ standard deviation. Assumptions of normality and homogeneity of variance were checked. Square root or log transformation was done for skewed data. An analysis of variance (ANOVA) was performed between groups of treatments for various parameters. A Bonferroni post-hoc test was done for multiple comparisons following the ANOVA. The level of significance was given at $p < 0.05$.

Table 1 Proximate major nutrients of standard diet and flaxseed supplemented diets.

Nutrient	SD	FLX ^a	SD+FLX (90:10)
Protein (g)	20	18.3	19.8
Fat (g)	3	42.2	6.9
Carbohydrate (g)	56.4	29	53.6
Fiber (g)	5.5	27.3	7.7
Water (g)	8.75	7.0	8.6
Energy (Kcal)	285	535	310

SD- standard diet, FLX- flaxseed diet, SD+FLX- flaxseed supplemented standard diet. ^aRef.13

3 RESULTS

3.1 Body weight, food and energy intakes

To assess the effects of food and energy intakes on body weight, these parameters were compared among the studied groups. Food intake (g/day) among the control, CYS and CYS + FLX groups was not significantly different at baseline (23 ± 1.8 , 22 ± 1.3 , and 22 ± 1.5 g/day, respectively; $p > 0.05$), at 4 weeks (23 ± 2.1 , 23 ± 1.4 , and 22 ± 1.8 g/day, respectively; $p > 0.05$) and at 8 weeks (23 ± 1.6 , 22 ± 1.7 , and 23 ± 1.5 g/day, respectively; $p > 0.05$). Energy intakes (Kcal/day) among the control, CYS and CYS + FLX groups was also comparable at baseline (65.5 ± 5.13 , 58.96 ± 3.7 , 68.2 ± 4.65 Kcal/day, respectively; $p > 0.05$), at 4 weeks (65.5 ± 5.98 , 65.5 ± 3.99 and 68.2 ± 4.65 Kcal/day, respectively; $p > 0.05$) and at 8 weeks (65.5 ± 4.56 , 58.96 ± 4.84 and 71.3 ± 4.65 Kcal/day, respectively; $p > 0.05$). Similarly, no significant change in the body weights (g) were observed between the control, CYS and CYS + FLX groups at baseline (525 ± 28 , 521 ± 31 and 522 ± 33 g, respectively; $p > 0.05$), at 4 weeks (521 ± 30 , 524 ± 29 and 525 ± 28 g, respectively; $p > 0.05$) and at 8 weeks (528 ± 33 , 520 ± 35 and 520 ± 34 g, respectively; $p > 0.05$) of the feeding duration.

3.2 Effect of the flaxseed diet on lipid profiles

Effects of the flaxseed diet on lipid profiles in the control,

CYS and CYS + FLX groups are presented in Table 2. Compared to rats in the control group, the CYS group had significantly elevated systolic and diastolic blood pressures, total cholesterol, TAG, and LDL-cholesterol with significantly decreased HDL-cholesterol at baseline, 4 and 8 weeks. These levels were comparable to those in the CYS + FLX group at baseline. However, the CYS + FLX group exhibited significantly decreased systolic and diastolic blood pressures, total cholesterol, TAG, and LDL-cholesterol along with significantly increased HDL-cholesterol levels after 4 and 8 weeks compared to time matched CYS group rats.

3.3 Effect of the flaxseed diet on AST and ALT

Effects of the FLX diet on AST and ALT are presented in Fig. 1. At baseline the AST and ALT levels were significantly elevated ($p < 0.05$ and $p < 0.01$, respectively) in the CYS and CYS + FLX groups compared to the control group. These levels remained significantly elevated ($p < 0.05$ and $p < 0.01$ respectively) when tested after 4 and 8 weeks in the CYS group compared to the time-matched control. Alternatively, these levels were significantly decreased ($p < 0.05$ and $p < 0.01$, respectively) after 4 weeks and remained significantly lower ($p < 0.05$ and $p < 0.01$, respectively) at 8 weeks in the CYS + FLX group compared to the time-matched CYS group.

Table 2 Lipid profile in control and hypertensive rats on standard or FLX supplemented diet.

Parameters	Duration	Control (N=10)	CYS (N=10)	CYS + FLX (N=10)	P value
SBP (mmHg)	Baseline	108.5 $\pm$ 18.1	130.8 $\pm$ 11.9 *	131.0 $\pm$ 21.7 *	<0.001
	4 weeks	106.0 $\pm$ 18.9	128.0 $\pm$ 13.1 *	116.0 $\pm$ 19.4 †	<0.001
	8 weeks	105.2 $\pm$ 17.5	125.7 $\pm$ 21.7 *	114.0 $\pm$ 17.3 †	<0.001
DBP (mmHg)	Baseline	67.6 $\pm$ 14.4	117.8 $\pm$ 21.7 *	90.0 $\pm$ 13.5 *	<0.001
	4 weeks	72.1 $\pm$ 21.0	113.0 $\pm$ 18.7 *	87.0 $\pm$ 11.9 †	<0.001
	8 weeks	67.7 $\pm$ 23.0	116.0 $\pm$ 15.6 *	93.0 $\pm$ 14.3 †	<0.001
Cholesterol (mmol/l)	Baseline	1.2 $\pm$ 0.24	2.3 $\pm$ 0.28*	2.2 $\pm$ 0.27*	<0.001
	4 Weeks	1.3 $\pm$ 0.25	2.4 $\pm$ 0.30*	1.7 $\pm$ 0.26†	<0.001
	8 Weeks	1.3 $\pm$ 0.23	2.4 $\pm$ 0.31*	1.6 $\pm$ 0.25†	<0.001
TAG (mmol/l)	Baseline	0.17 $\pm$ 0.05	0.32 $\pm$ 0.12*	0.35 $\pm$ 0.13*	<0.01
	4 Weeks	0.18 $\pm$ 0.06	0.33 $\pm$ 0.08*	0.24 $\pm$ 0.07†	<0.001
	8 Weeks	0.18 $\pm$ 0.07	0.35 $\pm$ 0.08*	0.23 $\pm$ 0.08†	<0.001
HDL-cholesterol (mmol/l)	Baseline	0.68 $\pm$ 0.06	0.45 $\pm$ 0.06*	0.43 $\pm$ 0.05*	<0.001
	4 Weeks	0.67 $\pm$ 0.07	0.43 $\pm$ 0.05*	0.58 $\pm$ 0.07†	<0.001
	8 Weeks	0.68 $\pm$ 0.07	0.42 $\pm$ 0.04*	0.60 $\pm$ 0.08†	<0.001
LDL-cholesterol (mmol/l)	Baseline	0.24 $\pm$ 0.05	0.55 $\pm$ 0.08*	0.56 $\pm$ 0.08*	<0.001
	4 Weeks	0.25 $\pm$ 0.06	0.56 $\pm$ 0.09*	0.35 $\pm$ 0.06†	<0.001
	8 Weeks	0.26 $\pm$ 0.07	0.55 $\pm$ 0.09*	0.32 $\pm$ 0.05†	<0.001

CYS-cyclosporine A; FLX-flaxseed; SBP- systolic blood pressure; DBP- diastolic blood pressure; TAG- triacylglycerol; *significantly different compared to control; †significantly different compared to CYS.

Fig. 1 Effects of a flaxseed-supplemented diet on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in hypertensive rats. Normotensive rats on a standard diet were rendered hypertensive with cyclosporin A. Hypertensive rats were either fed a standard diet alone or the flaxseed-supplemented standard diet (10% W/W) for 8 weeks. Blood samples from control (normotensive), CYS (hypertensive) and CYS+FLX (flaxseed-supplemented diet and hypertensive) rats were collected at baseline, 4 and 8 week intervals and plasma levels of AST and ALT were measured. * Significantly different from control ($p < 0.05$, $p < 0.01$ for AST and ALT, respectively), # significantly different from CYS ($p < 0.05$, $p < 0.01$ for AST and ALT, respectively).

3.4 Effect of the flaxseed diet on BUN and UA

The BUN and UA levels in the control, CYS and CYS + FLX groups are provided in Fig. 2. The BUN and UA levels were significantly increased at baseline in the CYS and CYS + FLX groups rats compared to the normotensive control group ($p < 0.001$ and $p < 0.01$, respectively). These levels remained significantly elevated in the CYS group at 4 and 8 weeks compared to the time-matched controls ($p < 0.001$ and $p < 0.01$, respectively). In contrast, rats in the CYS + FLX group showed significantly reduced BUN and UA levels after 4 and 8 weeks in comparison to time-matched levels measured in the CYS group ($p < 0.01$ and $p < 0.01$ respectively).

Fig. 2 Effects of a flaxseed-supplemented diet on blood urea nitrogen (BUN) and UA levels in hypertensive rats. Normotensive rats on a standard diet were rendered hypertensive with cyclosporin A. Hypertensive rats were either fed a standard diet alone or the flaxseed-supplemented standard diet (10% W/W) for 8 weeks. Blood samples from normotensive (control), hypertensive (CYS) and diet-supplemented hypertensive (CYS+FLX) rats were collected at baseline, 4 and 8 week intervals and plasma levels of BUN and UA were measured. * Significantly different from control ($p < 0.001$, $p < 0.01$ for BUN and UA, respectively), # significantly different from CYS ($p < 0.01$, $p < 0.01$ for BUN and UA, respectively).

spectively).

3.5 Effect of the flaxseed diet on creatinine and renin

Effects of the flaxseed on creatinine and renin levels are shown in Fig. 3. Compared to control rats, the CYS group demonstrated significantly increased creatinine and renin levels at baseline and thereafter at 4 and 8 week durations ($p < 0.01$, respectively). The levels of creatinine and renin in the CYS + FLX group were also comparable to baseline

Fig. 3 Effects of a flaxseed-supplemented diet on creatinine and renin in hypertensive rats. Normotensive rats on a standard diet were rendered hypertensive with cyclosporin A. Hypertensive rats were either fed a standard diet alone or the flaxseed-supplemented standard diet (10% W/W) for 8 weeks. Blood samples from normotensive (control), hypertensive (CYS) and diet-supplemented hypertensive (CYS+FLX) rats were collected at baseline, 4 and 8 week intervals and plasma levels of creatinine and renin were measured. * Significantly different from control ($p < 0.01$, $p < 0.01$ for creatinine and renin, respectively), # significantly different from CYS ($p < 0.05$, $p < 0.05$ for creatinine and renin, respectively).

levels found in CYS group rats ($p < 0.01$), however, these levels were significantly reduced after 4 weeks ($p < 0.05$) and remained significantly low even after 8 weeks ($p < 0.05$) compared to those in the time-matched CYS group.

4 DISCUSSION

Functional foods are considered superior in the prevention and treatment of several chronic diseases mainly due

to their reduced side effects. Flaxseed is an excellent source of oil, protein and dietary fibre as well as a rich source of the phytochemical antioxidant, lignan^{6, 7}. Flaxseed oil is rich in ALA and LA^{7, 8}. Fibre and lignan contents of flaxseed are reported to reduce both the total- and LDL-cholesterol levels¹⁸. Meanwhile, the ALA content of flax oil is suggested to be antiatherogenic because of its anti-inflammatory and antiproliferative properties^{19, 20}. Flaxseed gained further importance following the identification of its antioxidant property, which is essential in blocking oxidative stress, and is believed to be central to the initiation and progression of a majority of chronic diseases^{21–23}. Hypertension is a major risk factor of cardiovascular diseases and is also known to negatively impact various organs including the liver and kidneys. Several studies have reported the positive effects of flaxseed oil to normalize blood pressure and to diminish the level of hypertension^{10–12}. Considering that hypertension negatively impacts liver and kidney functions, the present study was undertaken to examine whether flaxseed, with its major essential fatty acid content, could alleviate the hypertension and its negative effects on liver and kidney functions.

In the present study, the increased blood pressure and altered lipid parameters in cyclosporine-treated rats confirms the hypertensive state and its unfavourable effects on lipid profiles. Significant reductions in the blood pressure and favourable improvements in lipid profiles following the supplementation of a flaxseed diet is consistent with the antihypertensive and antidyslipidemic characteristics of flaxseed or its individual components^{7, 10–12, 23–25}. The antihypertensive nature of flaxseed can be explained by several possible mechanisms. Angiotensin converting enzyme, which assists in regulating blood pressure, is inhibited by the ALA of flaxseed at both the phenotypic and genetic levels in spontaneously hypertensive rats (SHR)^{10, 21–23}. Likewise, supplementation of flaxseed oil lowered the blood pressure, body weight and adiposity in hypertensive rats¹¹. A flaxseed diet has also been shown to act as vaso-relaxant and restore the endothelial function in SHR²⁶. Hypertension may also increase several biochemical parameters associated with liver and kidney functions including AST, ALT, bilirubin, BUN, UA, creatinine and renin^{27, 28}. Consistent with the detrimental effects of hypertension on the liver and kidneys, cyclosporine-treated hypertensive rats in the present study demonstrated significantly increased levels of these functional markers compared to normotensive rats.

Several studies have previously shown the positive effects of flaxseed or its components on liver and kidney functions in various pathological conditions. For example, flaxseed oil significantly reduced the AST and ALT enzyme levels and ablated the non-alcoholic fatty liver in hamsters along with acute and chronic arthritis in albino rat models^{29, 30}. Similarly, flaxseed oil has been shown to lower the AST and

ALT in radiation-induced hepatotoxicity in mice²³⁾. Further, studies have reported the positive effects of flaxseed oil, or flaxseed per se, on kidney functions. Treatment with flaxseed oil significantly reduced the sodium nitroprusside and L-arginine-induced increase in BUN levels in the plasma and kidneys of rats^{31, 32)}. Flaxseed-supplemented diets as well as flax oil, markedly lowered the UA level in healthy rats and in the condition of renal toxicity^{33, 34)}. Additionally, flaxseed oil or flaxseed diets have lowered the serum and kidney creatinine levels in several pathological and experimental conditions^{31, 32, 35, 36)}. Flaxseed oil has been shown to inhibit the renin mRNA levels in SHR³⁷⁾. Collectively, these data clearly demonstrate the protective effects of flaxseed or flaxseed oil in improving the liver and kidney functions in various pathophysiological environments. Consistent with these observations, the present study shows that a flaxseed-supplemented diet significantly lowered the plasma levels of liver functional markers including AST and ALT and the kidney markers including BUN, UA, creatinine and renin in hypertensive rats. This suggests that flaxseed has the ability to not only attenuate hypertension but also to exert beneficial effects on hypertension-induced alterations in liver and kidney functions.

5 CONCLUSION

Flaxseed oil has been directly linked to restoring blood pressure and diminishes hypertension as well as restoring functional markers of liver and kidney, albeit in pathological conditions other than hypertension. It is likely that the ALA and LA content of flax oil, which is the major constituent of flaxseed, exerted these beneficial effects on liver and kidney functions in the present study. This is the first study to identify the favourable effects of the flaxseed-supplemented diet on liver and kidney function in a hypertensive setting as well as confirm its antihypertensive and antidyslipidemic effects. Thus, the results of this study would suggest that a regular consumption of diets rich in essential oils may lead to positive health effects.

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