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Research Article
Effect of Flaxseed on Lipid Profile, Antioxidants and PPAR-α Gene Expression in Rabbit Fed Hypercholesterolemic Diet

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Abstract

Background and Objective: The molecular explanation of hypocholesterolemic effect of flaxseed is not enough and needed further investigations. Therefore, the current study aimed to investigate its effect on lipid profile, antioxidants and peroxisome proliferator activated receptor alpha (PPAR-α) gene expression in hypercholesterolemic rabbits. **Materials and Methods:** One hundred rabbits were divided into 4 equal groups. Rabbits in the first group fed basal diet only and served as negative control. Rabbits in the second group fed basal diet mixed with 1% cholesterol and served as positive control. Rabbits in the third group fed basal diet mixed with 10% full fat flaxseed. Rabbits in the fourth group received a combination of second and third groups. Beside the estimated growth performance parameters, blood, liver and aorta samples were collected from each group at the end of the experiment (8 weeks) for determination of serum lipid profile, serum and hepatic oxidative stress bio-markers, gene expression of hepatic PPAR-α and histopathology of liver and aorta. **Results:** Flaxseed improved the disrupted growth performance parameters and histopathology picture of rabbits fed high cholesterol diet. Flaxseed induced significant decrease in the levels of total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein cholesterol (LDL-C) and expression of PPAR-α gene and antioxidant biomarkers in rabbits fed high cholesterol diet. **Conclusion:** These results suggested that the studied dose of flaxseed might be a potential protective therapy against hypercholesterolemia in rabbits.

Key words: flaxseed, PPAR-α gene, hypercholesterolemia, cholesterol diet, basal diet

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

INTRODUCTION

Hypercholesterolemia is one of the most excessively known as a predominant hazard factor for the development of cardiovascular diseases[1]. Hyperlipidemia is a reason for generation of reactive oxygen species (ROS) and creation of a state of oxidative stress in main organs, heart, kidney and liver[2]. The state of oxidative stress in the heart is ended by coronary heart disease and atherosclerosis[3]. Without a doubt, success lowering of serum cholesterol reduces coronary artery disease. Chemical drugs are used for lowering cholesterol, however higher price and undesirable side effects are the main disadvantages[4]. Currently, world attentions directed to the traditional medicine and uses of medicinal plants[5]. Flaxseed has a great role in the area of disease and diet investigation due to its beneficial effects to health and disease prevention[6]. Flaxseed used in protection against many chronic diseases and hazard factors as hyperlipoproteinemia, colon carcinogenesis and breast carcinogenesis, insulin dependent diabetes mellitus, atherosclerosis, cardiovascular diseases and related condition[7]. Flaxseed (*Linum usitatissimum*) considers a privilege source of protein with good-quality and soluble fiber and has great potency as a source of phenolic compounds, in addition to being a member of the highest sources of lingams lignans and 3 fatty acids[8]. Peroxisome proliferator-activated receptors (PPARs) are transcription factors that belong to the superfamily of nuclear hormone receptors and regulate the expression of several genes involved in metabolic processes that are potentially linked to the development of some diseases such as hyperlipidemia, diabetes and obesity[9,10]. The hepatic isoform of PPAR, PPAR-α is a transcription factor that regulates the metabolism of lipids, carbohydrates and amino acids. It activated by ligands such as polyunsaturated fatty acids and it is the target for the hypocholesterolemic drugs[9,10]. Moreover, activated PPAR-α is essential for the control of cellular development and body bioenergetics[11]. As indicated above, the protective effect of flaxseed against hypercholesterolemia has been documented. However, molecular explanation of its mechanism of action requires further investigations. Therefore, the current study aimed to investigate the effects of flaxseed-rich diet on lipid profile, antioxidant status and peroxisome proliferator activated receptor alpha (PPAR-α) gene expression in rabbits fed hypercholesterolemic diet.

MATERIALS AND METHODS

Animals and experimental protocol: One hundred apparently healthy White New Zealand male rabbits (7 weeks old, average body weight 1.3 kg) were obtained from Laboratory Animal House, College of Veterinary Medicine, King Faisal University, Saudi Arabia. The experiments performed during January and February, 2018. Rabbits were fed on basal diet for 1 week at the laboratory environment for acclimatization before being used. The laboratory relative humidity was 40-60% and temperature was 18-27°C, with photoperiod 16 h. The animals were housed individually in galvanized cages (90×60×40 cm) during the 8 weeks feeding period. The diets formulated and balanced to meet the nutrient requirement of the growing rabbit[12] as shown in Table 1. Rabbit were allowed free access to water. The handling and the maintenance of the animals were done according to guidance of King Faisal University from the Ethical Committee for Research on Laboratory Animals (Approval No. 186025). Rabbits were divided randomly into four equal groups:

- Rabbits in the first group fed basal diet only and served as negative control
- Rabbits in the second group fed basal diet mixed with 1% cholesterol and served as positive control
- Rabbits in the third group fed basal diet mixed with 10% full fat flaxseed
- Rabbits in the fourth group fed basal diet mixed with 1% cholesterol and 10% full fat flaxseed for 8 weeks

The full-fat flaxseed were grounded and mixed with other feed ingredients before pelleting. The pelleting process was carried out by pelting machine. The chemical analysis of feed ingredients including flaxseed was performed with near infrared spectroscopy (NIRS™ DS2500, FOSS analytical AB, Höganäs, Sweden) as illustrated in Table 1 and 2. Throughout the experimental period, live body weight, weight gain, feed consumption and feed conversion ratio were carried out weekly.

Sampling and analysis: By the end of the experiment, the overnight fasting rabbits in each group were anesthetized with 5 mg kg^{-1} ketamine and 25 mg kg^{-1} xylazine. Afterwards, two aliquots of individual blood samples were collected from the marginal ear vein puncture. The first aliquot was collected in EDTA vacutainers and rapidly centrifuged at 3000 g for 10 min and the obtained plasma was used for estimation of catalase (CAT) activity whereas, the erythrocyte lysate was used for determination of the activities of reduced glutathione concentration (GSH) and superoxide dismutase (SOD). The second blood aliquot was collected in plain vacutainers and the harvested sera were stored frozen at -80°C until the time of analysis of total cholesterol (TC),

Table 1: Ingredients and chemical composition of the experimental diets

	Groups			
Items	1	2	3	4
Ingredients (%)				
Corn (grain)	23.3	23.3	19.5	19.5
Flaxseed	0.0	0.0	10.0	10.0
Soybean (44%)	18.0	18.5	15.0	15.3
Wheat bran	16.0	16.0	10.5	10.5
Berseem hay	39.0	37.5	41.8	40.5
Cholesterol	0.0	1.0	0.0	1.0
Molasses	1.5	1.5	1.0	1.0
Limestone	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.3	0.3	0.3	0.3
[a]Premix	0.3	0.3	0.3	0.3
Common salt	0.5	0.5	0.5	0.5
DL-methionine	0.1	0.1	0.1	0.1
Total	100.0	100.0	100.0	100.0
Chemical composition (unless stated%)				
DM	90.55	89.77	89.83	89.95
[b]DE (kcal kg^{-1} DM)	2700.00	2700.00	2700.00	2700.00
Crude protein	18.00	18.00	18.00	18.00
Calcium	1.01	1.00	1.06	1.05
Phosphorus	0.54	0.54	0.53	0.53
Organic matter	83.31	82.52	82.35	82.55
Ash	7.24	7.25	7.48	7.39
NFE	51.26	50.90	45.68	46.23
EE	2.45	2.44	5.38	5.37
Crude fiber	12.38	12.04	13.16	12.86
Methionine+cystine	0.80	0.68	0.74	0.74

Ingredient and nutrient composition are reported on as-fed basis. [a]The vitamin and mineral premix provided per kg of diet: Vitamin A, 4000000 IU, Vitamin D3, 667000 IU, Vitamin E 3334 mg, Vitamin K3, 1167 mg, Vitamin B1, 334 mg; Vitamin B2, 1667 mg; Vitamin B3, 3334 mg; B6, 500 mg; Vitamin B12 33.4 mg, Folic acid, 334 mg; Biotin, 17 mg; Iron, 10; Copper, 2.167; Zinc, 18.334; Manganese 20.0; Iodine, 0.167; Cobalt, 0.034 and Selenium, 0.034, [b]Calculated based on NRC[12] feed composition tables. DM: Dry matter, DE: Digestible energy, NFE: Nitrogen fee extract, EE: Ether extract. Group 1: Negative control (basal diet only), Group 2: Positive control (basal diet+1% cholesterol), Group 3: Basal diet+flaxseed 10%, Group 4: basal diet+1% cholesterol+10% flaxseed

Table 2: Proximate analysis of full fat flaxseed (fed basis %)

Nutrients	Percentage (%)
Dry matter	91.5
Crude protein	23.0
Crude fat	33.0
Crude fiber	9.0
Ash	4.3
Nitrogen free extract	22.2
Organic matter	87.2

triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and malondialdehyde (MDA) levels. The selected biochemical variables were analyzed using commercial assay ELISA kits (Cayman Chemical, Ann Arbor, Michigan, USA) according to the instruction manual. After collection of the blood samples rabbits were sacrificed and the liver and the aorta tissues have been collected. The collected tissues were washed with ice-cold normal saline and divided into two parts. The first part was processed routinely for histopathological examination. The second part was used to prepare tissue homogenates (10% weight/volume) in phosphate buffered saline (pH 7.4). The tissue homogenates were divided into 2 aliquots. The first aliquot was centrifuged in a cooling centrifuge at 5000 rpm for 10 min at 4°C and the obtained supernatant was stored frozen at -80°C until the time of estimation of MDA, NO, GSH, SOD and CAT levels using commercial ELISA kits (Cayman Chemical, Ann Arbor, Michigan, USA) according to the manufacture instructions. The second aliquots of the homogenate were used for estimation of mRNA level of PPAR-α gene.

Quantification of mRNA of the respective genes by RT-PCR: Total RNA from liver samples was extracted according to the instruction of purification kit (RNeasy Mini RNA extraction kit, Qiagen, USA). About 2 µg of total RNA for each sample was reverse transcript into cDNA using Revert Aid Reverse Transcriptase kit (Thermo Fisher Scientific, California, USA). Sequences of primers used for housekeeping (GAPDH) and tested gene expression shown in Table 3. The amplification of cDNA was done using Luminaris Color HiGreen qPCR Master Mix (Thermo Scientific, USA)[13].

Histopathological studies: Liver and aorta of dissected tissues of different group fixed in 10% neutral buffered formalin for histopathological examination. Afterwards, the sections stained by hematoxylin and eosin (H and E, Sigma Aldrich, USA) and examined microscopically as described earlier by Bancroft and Gamble[14].

Statistical analysis: The results obtained were analyzed using SPSS version[15] 16.0. The Kolmogorov Smirnov test was used to test the normal distribution of the data. The data was expressed by mean accompanied with its standard error and statistically analyzed for significance using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test at a significant[16] level of $p \leq 0.05$. The data represented as the mean±standard error.

RESULTS

Growth performance parameters: The data summarized in Table 4 indicated that, final body weight, daily feed intake, total weight gain and daily weight gain were decreased ($p<0.001$) in rabbits fed high cholesterol diet alone compare to negative control group. Inclusion of flaxseed 10% only in the diet of rabbits induced a significant increase ($p<0.001$) in the final body weight, total weight gain and daily weight gain as compare to other groups including the negative control. Final body weight, daily feed intake, total weight gain, daily weight gain and feed conversion ratio were significantly increased in rabbits fed high cholesterol diet mixed with flaxseed 10% compare to that of rabbits fed high cholesterol diet alone and was close to negative control values.

Lipid profile: As indicated in Table 5, the serum concentrations of TC, TAG and LDL-C were significantly increased ($p<0.001$) whereas, the serum concentration of HDL-C was significantly decreased ($p<0.001$) in rabbits fed high cholesterol diet without treatment compare to negative control. However, the concentrations of serum TC, TAG and LDL-C were decreased in the serum of rabbits fed high cholesterol diet mixed with flaxseed 10% compare to that fed high cholesterol diet alone and nears to the control values (Table 5). The serum concentrations of TC, TAG, LDL-C and HDL-C in the serum of rabbits fed basal diet mixed with flaxseed 10% were unchanged significantly when compare to that of negative control group (Table 5).

Serum and hepatic oxidative stress bio-markers and antioxidants: The current findings (Table 6) showed that, the serum and hepatic concentrations of MDA were significantly increased ($p<0.001$) whereas, the serum and hepatic activities of CAT and SOD and concentration of GSH were significantly decreased ($p<0.001$) in rabbits fed high cholesterol diet alone compare to negative control. However, the concentrations of serum and hepatic MDA were decreased and the serum and hepatic activities of CAT and SOD and concentration of GSH were increased in the rabbits fed high cholesterol diet mixed with flaxseed 10% compare to that fed high cholesterol diet alone (Table 6) and were close to negative control values. The serum and hepatic concentrations of MDA and GSH in addition to the serum and hepatic activities of CAT and SOD of rabbits fed basal diet mixed with flaxseed 10% were unchanged significantly when compare to that of negative control (Table 6).

Table 3: Primer sequences for gene expression by real-time PCR

Gene name	Primer sequence	Accession No.
GAPDH	F: 5'TGGTGAAGGTCGGAGTGAAC3'	NM:001082253
	R: 5'ATGTAGTGGAGGTCAATGAATGG3'	
PPAR- α	F: 5'CCTGGCCTTCTAAACATAGGAT3'	NM:002723354
	R: 5'TGTAGATCTCTTGCAACAGTGG3'	

GAPDH: Glyceraldehyde-3-Phosphate dehydrogenase, PPAR-α: Per-oxisome proliferator activated receptor alpha

Table 4: Growth performance parameters of rabbits fed high cholesterol diet (1%) and treated with flaxseed 10%

Items	Group 1	Group 2	Group 3	Group 4	p-value
Initial body weight (g)	1309.10±4.50	1308.00±4.42	1306.70±2.32	1309.40±2.12	0.95
Final body weight (g)	2933.00±5.73^{b}	2758.10±3.66^{d}	3222.50±8.79^{a}	2903.20±2.98^{c}	<0.001
Daily feed intake (g)	111.80±1.63^{a}	90.80±0.98^{c}	108.00±1.58^{ab}	106.10±1.28^{b}	<0.001
Total weight gain (g)	1623.90±7.18^{b}	1450.10±5.75^{d}	1915.80±10.46^{a}	1593.80±3.78^{c}	<0.001
Daily weight gain (g)	27.06±0.12^{b}	24.16±0.09^{d}	31.93±0.170^{a}	26.56±0.06^{c}	<0.001
Feed conversion ratio	4.13±0.07^{a}	3.76±0.05^{b}	4.38±0.050^{c}	3.99±0.05^{a}	<0.001

Values are expressed as Mean±SE, Means bearing different superscript letters in the same row differ significantly at the corresponding p-value. Group 1: Negative control (basal diet only), Group 2: Positive control (basal diet+1% cholesterol), Group 3: Basal diet+flaxseed 10%, Group 4: Basal diet+1% cholesterol+10% flaxseed

Table 5: Serum lipid profile of rabbits fed high cholesterol diet (1%) and treated with flaxseed 10%

Items	Group 1	Group 2	Group 3	Group 4	p-value
TC (mg dL^{-1})	81.91±9.77^{c}	303.27±4.21^{a}	74.63±2.18^{c}	187.27±3.76^{b}	<0.001
TAG (mg dL^{-1})	91.20±11.53^{b}	281.88±38.84^{a}	74.82±2.29^{b}	105.00±7.20^{b}	<0.001
HDL-C (mg dL^{-1})	20.63±2.52^{ab}	18.83±1.71^{b}	25.85±1.45^{a}	18.15±1.98^{b}	<0.050
LDL-C (mg dL^{-1})	43.04±7.22^{c}	228.05±11.33^{a}	33.82±2.46^{c}	148.12±3.95^{b}	<0.001

Values are expressed as Mean±SE. Means bearing different superscript letters in the same row differ significantly at the corresponding p-value. TC: Total cholesterol, TAG: Triacylglycerol, HDL: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein cholesterol, Group 1: Negative control (basal diet only), Group 2: Positive control (basal diet+1% cholesterol), Group 3: Basal diet+flaxseed 10%, Group 4: basal diet+1% cholesterol+10% flaxseed

Table 6: Oxidative stress bio-markers and selected anti-oxidant enzymes activities in blood and tissue of rabbits fed high cholesterol diet (1%) and treated with flaxseed 10%

Parameters	Group 1	Group 2	Group 3	Group 4	p-value
Blood metabolites					
MDA (nmol mL^{-1})	15.78±1.27^{c}	60.75±2.78^{a}	14.20±0.76^{c}	49.7.00±2.7^{b}	<0.001
CAT (U L^{-1})	795.00±13.35^{a}	469.67±16.46^{c}	794.45±18.52^{a}	725.00±20.45^{b}	<0.001
SOD (U mL^{-1})	341.88±0.84^{a}	252.34±1.58^{c}	340.97±5.47^{a}	307.97±1.52^{b}	<0.001
GSH (mg dL^{-1})	4.67±0.43^{a}	2.68±0.35^{b}	4.73±0.49^{a}	4.03±0.19^{a}	<0.001
Liver metabolites					
MDA (nmol g^{-1})	13.03±0.9^{c}	63.73±2.60^{a}	12.57±0.78^{c}	29.75±3.85^{b}	<0.001
CAT (U g^{-1})	0.89±0.02^{a}	0.46±0.11^{b}	0.92±0.01^{a}	0.73±0.12^{a}	<0.001
SOD (U g^{-1})	319.75±2.65^{a}	264.44±1.37^{c}	319.74±4.63^{a}	283.97±1.17^{b}	<0.001
GSH (mmol g^{-1})	4.92±0.37^{a}	3.75±0.52^{b}	5.07±0.40^{a}	4.08±0.62^{a}	0.192

Values are expressed as Mean±SE. Means bearing different superscript letters in the same row differ significantly at the corresponding p-value. MDA: Malondialdehyde, CAT: Catalase, SOD: Superoxide dismutase, GSH: Glutathione peroxidase. Group 1: Negative control (basal diet only), Group 2: Positive control (basal diet+1% cholesterol), Group 3: Basal diet+flaxseed 10%, Group 4: Basal diet+1% cholesterol+10% flaxseed

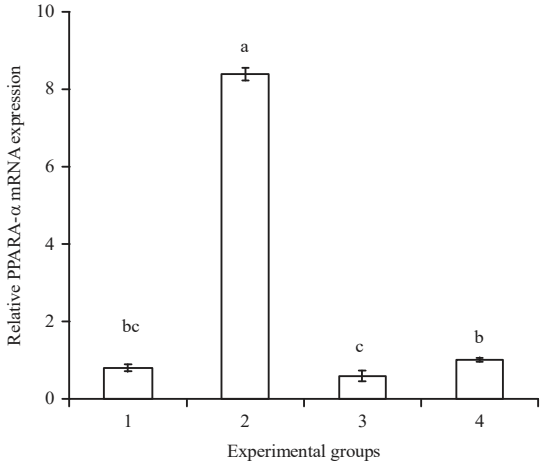

Fig. 1: Effect of different treatments on hepatic PPARA gene expression

Bars bearing different superscript letters differ significantly (p<0.05)

Relative mRNA expression of hepatic PPAR-α gene: The current study (Fig. 1) showed that, the relative mRNA expression of PPAR-α gene highly up regulated significantly (7 fold) in the liver of rabbits fed high cholesterol diet alone compare to negative control. However, the relative mRNA expression of PPAR-α gene down regulated significantly in the liver of rabbits fed high cholesterol diet mixed with flaxseed 10% compare to that fed high cholesterol diet alone (Fig. 1). The down regulation of effect of flaxseed on mRNA expression of PPAR-α gene was more pronounced when the seed mixed with basal diet rather than its mixing with high cholesterol and was comparable to that of negative control value.

Histopathological examination of aorta and liver: In the present study, in comparison with negative control (Fig. 2a) the histopathological examination showed atheromatous plague in tunica intima of aorta of rabbits fed high cholesterol diet (Fig. 2b). This plague formed from aggregations of foamy macrophages in sub-endothelial tissue (Fig. 2c). Aorta of rabbit fed high cholesterol diet (1%) and treated with flaxseed 10% showed some degree of recovery in the atheromatous plague in tunica intima and lower aggregation of foamy macrophages (Fig. 2d). The histopathological examination of the liver tissues in the current study revealed that, liver of negative control rabbits (group 1) and rabbits fed flaxseed 10% (group 3) showing normal hepatocytes and normal radial arrangement of hepatocytes (Fig. 3a). Liver of positive control rabbits (group 2, basal diet +1% cholesterol) showing severe degenerative changes of hepatocytes (Fig. 3b). Liver of rabbit fed high cholesterol diet (1%) and treated with flaxseed 10% showing some degree of recovery and regenerating hepatocytes with normal cell plates (Fig. 3c).

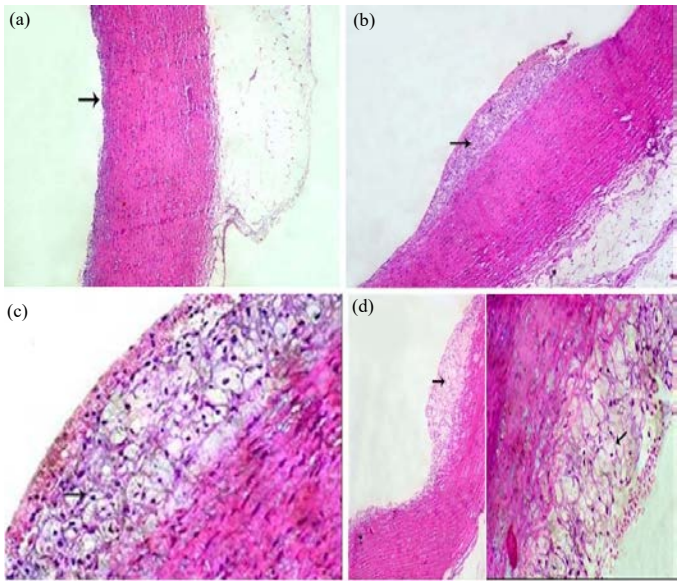

Fig. 2(a-d): Histopathological images of Rabbit Aorta fed high cholesterol diet (1%) and treated with flaxseed 10% (a) Aorta of (group 1) and (group 3) showing normal tunica intima (arrow), normal endothelium and normal tunica muscularis, (b) Aorta of positive control rabbits (group 2) showed atheromatous plague in tunica intima (arrow), (c) Aorta of positive control rabbits (group 2) showed atheromatous plague formed from aggregations foamy macrophages in sub-endothelial (arrow) and (d) Aorta of group 4 rabbit showed some degree of recovery in the atheromatous plague in tunica intima and lower aggregation of foamy macrophages (arrow)
HE bar = 100 µm

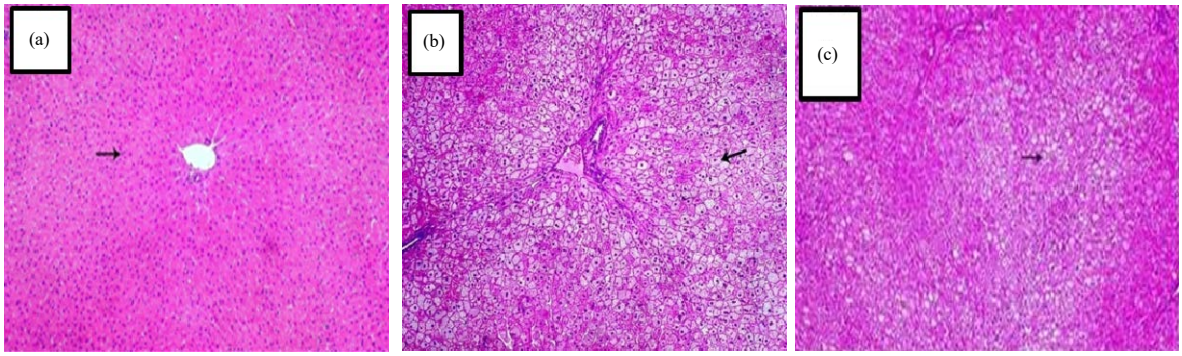

Fig. 3(a-c): Histopathological images of Rabbit liver fed high cholesterol diet (1%) and treated with flaxseed 10% (a) Liver of negative control rabbits (group 1) and rabbits fed flaxseed 10% (group 3) showing normal hepatocytes and normal radial arrangement of hepatocytes (arrow), (b) Liver of positive control rabbits (group 2, basal diet +1% cholesterol) showing severe degenerative changes of hepatocytes (arrow), (c) Liver of rabbit fed high cholesterol diet (1%) and treated with flaxseed 10% showing some degree of recovery and regenerating hepatocytes with normal cell plates (arrow)
HE bar = 100 µm

DISCUSSION

The high cholesterol diet induced a negative effect on daily feed intake, body weight gain and final body weight. In addition, it reduced the efficiency of feed conversion to meat in rabbits under present experimental condition. These results are consistent with earlier studies by Yanni[17] and Cha *et al.*[18]. It has been established that high cholesterol diet increase the

absorption of cholesterol in rabbits which induce hypercholesterolemia and animal become more susceptible to atherosclerotic plaques in the aorta which decrease blood flow and enhance the occurrence of cardiovascular disease[19]. The significant increase in final body weight, total weight gain and daily weight gain and the significant decrease in feed conversion ratio as a result of inclusion of flaxseed 10% either alone or as a mixture with high cholesterol diet in rabbits are in consistent with previous reports by Peiretti and Meineri[20] and Tariq *et al.*[21]. The growth promoting effect of flaxseed could be attributed to their nutritional values because it contains all essential amino acid[22]. Moreover, it is an excellent source of fiber, lecithin, vitamins, minerals and essential fatty acid like alpha-linolenic acids[22]. The significant increase in the serum concentrations of TC, TAG and LDL-C and the significant decrease in the serum concentration of HDL-C in rabbits fed high cholesterol diet without treatment compare to negative control parallel to the earlier reports in rabbits[23,24] and in rats[5,25] fed on the same cholesterol diet. This effect ended by production of atheromatous plague in tunica intima of aorta as a result of aggregations of foamy macrophages in sub-endothelial and severe degenerative changes of hepatocytes as clear in histopathological analysis of the current study. The observed significant decrease in the serum concentrations of TC, TAG and LDL-C in rabbits fed high cholesterol diet mixed with flaxseed 10% compare to that fed high cholesterol diet alone confirmed the hypolipidemic effect of flaxseed[26], however, it disagreed with previous reports in rabbits[27]. The earlier work by Lee and Prasad[27] suggested that, flaxseed oil does not produces an alteration in serum lipid or in the extent of hypercholesterolemic atherosclerosis. The hypocholesterolemia induced by flaxseed supported by the histopathology of aorta and liver that indicated a sort of recovery in the atheromatous plague in tunica intima and lower aggregation of foamy macrophages in addition to less degenerative changes to hepatocytes. Atherosclerosis suppression is associated with decreases in both serum lipids and oxidative stress biomarkers[28] and it was the reason behind the determination of oxidative stress biomarkers in the current study. The current significant increased in serum and hepatic concentrations of MDA in rabbits fed high cholesterol diet alone compare to negative control agrees with previous reports by Khaleel *et al.*[29] and El-Sayed *et al.*[30], whereas the observed significant decrease of serum and hepatic activities of CAT and SOD in the rabbits fed high cholesterol diet alone compare to negative control agreed with earlier report by Olorunnisola *et al.*[31]. The marked decrease in antioxidants and significant increase in MDA were indicators of decreasing the antioxidant defense system[32-35]. The decrease in the activities of studied antioxidant enzymes could be ascribed to the excessive utilization of these enzymes in deactivating the free radicals generated due to the hypercholesterolemic diet or inadequate availability of GSH[27,36]. The detected significant decrease in serum and hepatic MDA and GSH in addition to significant increase in hepatic activities of CAT and SOD of rabbits fed high cholesterol diet mixed with flaxseed 10% compare to that fed high cholesterol diet alone confirmed the antioxidant potential of flaxseed constituents (Lignans, ω-3fatty acids content and/or secoisolariciresinol diglucoside)[37]. The reduction of MDA concentrations in serum and tissues of rabbits fed high cholesterol diet mixed with flaxseed 10% came secondary to the reduction of LDL-c which is the main power for lipid peroxidation and MDA production[38] and subsequent atherosclerotic lesions[39]. The highly significant upregulation (7 fold) of hepatic PPAR-α gene expression in the liver of rabbits fed high cholesterol diet alone compare to negative control suggested the role of high cholesterol as a signaling power for upregulation of PPAR-α gene expression like that do of fatty acids[40,41]. This hypothesis supported by earlier findings which indicated an up-regulation of PPAR-α gene expression in hypercholesteremic rat as a result of extra cholesterol in the diet[42]. The highly significant upregulation (7 fold) of hepatic PPAR-α gene expression in the liver of rabbits fed high cholesterol diet alone compare to negative control may the reason behind the detected oxidative stress and lower antioxidant activities[43]. The downregulation of effect of flaxseed on mRNA expression of PPAR-α gene supported the biochemical and histopathological results mentioned above and potentiate the hypocholesterolemic effect of studied dose of flaxseed.

CONCLUSION

The current study can concluded that, flaxseed 10% improved growth performance, regulated the lipid profile, counteracted the oxidative stress, stimulated the activities of antioxidant enzymes and restored the expression of hepatic PPAR-α gene in rabbits fed high cholesterol diet. Therefore, flaxseed might be a potential protective therapy against hypercholesterolemia in rabbits.

SIGNIFICANCE STATEMENT

This study suggests a new evidence about the mechanism of action of hypocholesterolemic effect of flaxseed representing in downregulation to peroxisome proliferator activated receptor alpha (PPAR-α) gene expression in liver

tissues. The current study indicated that, flaxseed supplementation improved the disrupted growth performance parameters and histopathological picture in rabbits fed high cholesterol diet. The current study confirmed the hypocholesterolemic effect of flaxseed by regulation of lipid profiles, reduction of lipid peroxidation and enhancement of antioxidant enzymes activities. This study will help the researchers to explore important aspect of the potential molecular mechanisms of hypocholesterolemic effect of flaxseed thereby, aiding in further researches into the treatment of atherosclerosis. Thus, a new theory on flaxseed and atherosclerosis perhaps arrived at.

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