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REDUCTION OF SERUM HYPERCHOLESTEROLENIC IN MALE RABBITS BY SECOISOLARICIRESINOL DIGLUCOSIDE (LIGNAN) ISOLATED FROM FLAXSEED

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ABSTRACT
Secoisolariciresinol diglucoside (SDG, Lignan) is a plant lignan isolated from flaxseed may exert anti-inflammatory effects via its action as an antioxidant. Rabbits were assigned to 5 groups: group 1, normal diet; group 2, 2%cholesterol diet for 60days ,group 3, 2%cholesterol diet for 60days and then gave 40mg/ml partiall lignan ; group 4, same as group 3 but with added pure lignan and group 5, same as group 3 but with adminstation galantamine subcutaneous (injection).Blood samples were collected before (time 0) and after 60days and 74 days of experimental diets for measurement of serum triglycerides, total cholesterol (TC), and LDL, HDL, and VLDL cholesterol (LDL-C, HDL-C, and VLDL-C). Serum TC, LDL-C, and TG increased in Positive groups at 60days compared with time 0 days, but changes were lower in group 3 than in group 4 and 5 after treatment with lignan compound and galantamine. Pure lignan reduced TC, TG and LDL-C at 74 days from these results it can be concluded that flaxseed contains active components (lignans) which decrease serum lipid profile and lowers the risk of atherosclerosis in cholesterol fed rabbits may be through the inhibition of lipid peroxidation and through antioxidant activity.

KEY WORDS: Galantamine, Secoisolariciresinol diglucoside (SDG Lignan), Serum triglycerides

INTRODUCTION

Flaxseed (Linum usitatissimum L.) is an ancient crop with a long history of cultivation. It was grown in the earliest agrarian societies in the Tigris and Euphrates valleys in Mesopotamia around 6000 B.C. (Oates, 1979). Secoisolariciresinol diglucoside (SDG) is present in flaxseed in the form of complex polymeric structures, in which is covalently bound via ester linkages to 3-hydroxy-3-methyl glutaryl (HMG). SDG is the main exogenous precursor of the mammalian lignans, enterodiol and enterolactone. SDG is a recognized nutraceutical; it has been shown to reduce the levels of LDL-cholesterol (the bad cholesterol) in blood, the risk of diabetes, and hormone related cancer; it has antioxidant activity, cardioprotective effect, and improves renal function in lupus nephritis patients (Westcott and Muir, 2003a), Also, the flaxseed lignan, secoisolaricresinol diglucoside (SDG), may exert anti-inflammatory effects via its action as an antioxidant (Prasad, 2005).

The main objectives of this study are to determine whether lignan compound would reduce lipid levels in serum. An investigation was, therefore, made of the effects of high-cholesterol diet in rabbits then gave them (partial and pure) lignan and galantamine on the serum lipid profile [triglycerides (TG), total cholesterol (TC),high density lipoprotein-cholesterol (HDL-C), low density lipoprotein-cholesterol (LDL-C)].

MATERIALS AND METHODS

Preparation of flaxseed extract:

Firstly, cleaning flax from derbies, Secondly grinding flaxseeds by a grinder machine eventually obtained on a homogenized powder that was ready for extraction then defatting of flax oils by using Soxhelt apparatus according to (AAOC, 1984). So (50) g. of powder was put in a thumble and added (300) ml of petroleum ether was added within (40-60) ^{0}C for (6) hrs time when over, petroleum ether was substituted by Chloroform with the same volume and the same time, continuously on the same programme.,taking (25)g of defatted powder treats with a mixture of Dioxan and Ethanol alcohol (1:1), (v:v), respectively, with a ratio (1:8), (w:v), sample put on magnetic stirr for (4)hrs., then filtrated. The solvent was evaporated by rotary evaporator at (40) ^{0}C to obtain crud lignan**,** dissolving a certain amount of dried crud lignan in an alkaline hydrolysis solution (a methanolic NaOH, 20 mM, pH=8) at 50 ^{o}C for hydrolyzing SDG oligomers. The mixture was filtered then supernatant was concentrated with a rotary evaporator within (45) ^{o}C. Eventually, a thick sticky texture material, pH was corrected into 3.0 through adding drops of sulfuric acid 2 molar then the sample was stored in (4) ^{0}C.

Purification of Lignan

Amount of lignan (SDG) crude dissolve in Ethyl Acetate: distilled water ratio (1:7) .The mixture was obtained as in with doubled volume of separative system and using separating funnel the mixture was shaked well several times and until settled. Two layers were formed and make aqueous layer and the aqueous layer was concentrated with the rotary evaporator at (45) ^{0}C, (Li et al., 2008) nearly, drying amount of dried extract dissolved in 80 ml of ethanol and mixed with amount of silica gel (GF 254). After dried, this mixture was subjected to a column chromatography ,open glass(tube 3.5 x 30) cm and washed with 100 ml of ethyl acetate/ethanol (8.5:1.5, v/v), and then, SDG adsorbed by silica gel

was eluted with 180 ml of ethyl acetate/ethanol (8:2, v/v). two fractions (5 ml each) were collected and detected by, TLC and HPLC. The pure lignan was identified by (HPLC) according to (6), using ODS- reverse phase column and an elution system consisting of water/acidic, acidic /methanol under gradient condition of 100% aqueous acidic acid to 40% acidic acid /60% methanol .the lignan thus eluted were found to be purified to a level of greater than 90% in terms of physical,structural analysis consistent with known literature values.

Animals

Data are reported for a total of 20 male Specific Pathogen Free (SPF) wild type rabbits .Rabbits were 4 months old at the beginning of the study and weighed a mean of 1.5 kg. All rabbits were purchased from Abu Graib Market. Rabbits were individually housed in plastic cages in temperature and humidity controlled rooms. The light/dark cycle was 12/12-h.

Dietary Treatment

20 of the rabbits have been used to study the possible effect of lignan compounds of flaxseed effect compared to cholesterol -induced brain damage allocated as follows: **Group I –** four rabbits fed 160 g/day of a diet (pellet) without cholesterol for 74 days. **GroupII–** four rabbits were fed 160 g/day of a diet (pellet) contain 2% cholesterol for 60days **.,Group III –**four rabbits were fed 160 g/day of a diet (pellet) contain 2% cholesterol for 60 days then gave 40mg/kg/day of partial pure flax lignan for 14 days, **Group** IV**–** four rabbits were fed 160 g/day of a diet (pellet) contain 2% cholesterol for 60 days then gave 40mg/kg/day of pure flax lignan for 14 day, **Group** V**–** four rabbits were fed 160 g/day of a diet (pellet) contain 2%cholesterol for 60 days then gave 1.5mg/kg of galantamine S.C.(subcutaneous) for 14 days.

Methods of Biochemical Analysis of Blood Samples

After overnight fasting, all animals were subjected to cardiac puncture to collect blood for serum lipid profiles examination. The blood sample tubes were centrifuged at 3000 rpm for 2-5 minutes then the serum extracted to be stored or used immediately in the process of lipid profiles assay. (Total cholesterol, high density lipoprotein, low density lipoprotein and triglycerides were determined. the serum levels were obtained from animals at (0, 60, 72) days of feeding. Serum samples were analyzed for total cholesterol (Flegg, 1973), HDL-cholesterol (Wahlfeld, 1974) and triglycerides (Friedewald *et al*., 1972). LDL cholesterol and VLDL-cholesterol were calculated by method of (Dunean *et al*., 1983). Serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein cholesterol (HDL-C) were determined by colorimetric analysis with commercial kits (Biommaghreb) according to the manufacturer directions.

Statistical analysis

All the experiments were performed with di duplicate measurements and the results are shown as the mean ± S.E. Statistical analysis of data was performed with repeated measures analysis of variance (ANOVA) and $P \leq 0.05$ were considered to be significant also use LSD and SPSS statistical program , (Kivipelto and Solomon, 2006)**.**

RESULT AND DISCUSSION

Table 1. shows the result of mean value of serum cholesterol level (mg/dl) before and after treatment. Cholesterol-treatment group)positive control group, groupII (has shown significant ($P \leq 0.05$) increase in mean value of serum cholesterol level (1169.5mg/dl) compared to negative control group (group I) amount of (55mg/dl). Partial pure lignan-treatment (group III) have shown significant ($P \leq 0.05$)decrease in mean value of serum cholesterol level(143.8 mg/dl) compared to positive control group (group III) amount of(821.7mg/dl) .Pure lignan-treatment(group IV)have shown significant($P \leq 0.05$) decrease in mean value of serum cholesterol level(45mg/dl) compared to positive control group (group IV)amount of (1012.5mg/dl). Galantamine-treatment (group V) has shown significant ($P \leq 0.05$) decrease in mean value of serum cholesterol level (48.3 mg/dl) compared to compare to positive control group (groupV) amount of (1077.5mg/dl). These high increase in mean value of cholesterol level through the risk rang of Alzheimer disease have been agreed with Kivipelto and Solomon (12).who shows that the high total cholesterol at midlife is a risk factor for AD, in cholesterol-fed rabbits, increased serum total cholesterol level may be due, in most parts, to increased chylomicron remnant and / or β-VLDL cholesterol, which might be involved in the promotion of atherosclerosis (Mehta *et al*.,2003). These results are also supported by many earlier studies (Purohit and Vyas, 2006; Razay *et al*., 2007). Cholesterol added to the diet of the rabbit can result in the development of AD neuropathology.

Differently letters small or capital indicated there is significant difference ($p \leq 0.05$). Horizontal Significant difference represented by capital superscripted and vertical Significant difference represent by small superscripted. Time represent:0 days: normal diet(negative Control); 60days:2% cholesterol diet for all groups except group I (Control positive);74 days: treatment with partial pure ,pure lignan and galantamine diet for 14 days after 60days of cholesterol diet according to groups treatment program, …..=scarified animal.

Table 1. The results of mean serum total cholesterol levels (gm/dl) for rabbit after treatment.

Parameter	Experimental Groups	Treatment periods		
		0 days	60days	74days
Total cholesterol mg/dl	Group I	55±2.1 $^{a, A}$	55±2.1 ^{a,C}	55±2.1 $^{,a, B}$
	Group II	55±2.4 $^{b, A}$	1169.5±40.1 $^{a, A}$	-------
	Group III	53.8±1.5 $^{c, A}$	821.7±39.3 $^{a, B}$	143.8±13.9 ^{b,A}
	Group IV	49.4±0.6 $^{b, A}$	1012.5±79.4 $^{a, A}$	45±4 $^{b, B}$
	Group V	47.8±2.4 $^{b, A}$	1077.5±109.1 $^{a, A}$	48.3±1.9 $^{b, B}$

Each value represents mean ±SE

The decrease in mean value of serum cholesterol level of both lignan compounds treated rabbits have been agreed with Prasad (2005) who reported that flax lignan shows a significant reduce change in cholesterol level of rabbits treated with a dose of 40 mg/kg body weight daily orally for 60 days .

The cholesterol lowering effect of the plant extract is possibly associated with a decrease in intestinal absorption of cholesterol resulting in an increase in fecal excretion of neutral lipids.(Razay *et al*., 2007).or may be associated with a reduce in the level of lipid peroxide (TBARs).In aorta of cholesterol fed rabbits that uses as indicate enhanced oxidative stress in hyperlipidemic state which implicates in development and progression atherosclerotic lesions in aorta (Amin *et al*., 2011).Administration of plant extract decreased lipid peroxidation in these tissues indicating antioxidant like activity which alleviates oxidative stress.

Table 2. show the result of mean value of serum triglyceride level (mg/dl) before and after treatment. Cholesterol-treatment (positive control group,group II) have shown significant(P≤0.05)increase in mean value of serum triglyceride level(147.5 mg/dl) compared to negative control group(group I) amount of (44 mg/dl). Partial pure lignan-treatment (group III) has shown significant (P≤0.05) decrease in mean value of serum triglyceride level (56.5 mg/dl) compared to positive control group (group III) amount of (122 mg/dl). Pure lignan-treatment (group IV) has shown significant (P≤0.05) decrease in mean value of serum triglyceride level (50.8 mg/dl) compared to positive control group (group IV) amount of (255.8 mg/dl). Galantamine-treatment (group V) has shown a significant (P≤0.05) decrease in mean value of serum triglyceride level (51.3 mg/dl) compared to positive control group (group V) amount of (125.3 mg/dl).

These high increases in mean value of triglyceride level through the risk range of Alzheimer disease have been agreed with Razay *et al.*(2007) who show that high total triglyceride at midlife is a risk factor for atherosclerosis, Alzheimer disease and could at least partly explain the vascular changes in the brains of patients with AD. These results have been agreed with Amin *et al*, (2011) who found that Rats fed high fat diet for 12 weeks showed a significant increase in the lipid profile LDL and TG, also Kempaiah and Srinivasan (2006) found that Plasma triglyceride was elevated by about 85–90% in high-fat fed rats(30%) for 8 weeks.

Table 2. The effect of lignan compounds and galantamine on mean serum triglyceride (mg/dl) after treatment.

Experimental Groups	Treatment periods		
	0 days	60days	74days
Group I	44 ±1.3 ^{a,A}	44 ±1.3 ^{a,C}	44 ±1.3 ^{a,A}
Group II	47.3±2.2 $^{b, A}$	147.5±4.9 $^{a, B}$	……
Group III	43±1.8 $^{b, A}$	122±6.2 $^{a, B}$	56.5±8.8 $^{b, A}$
Group IV	41.5±1.2 $^{b, A}$	255.8±80.2 $^{a, A}$	50.8±4.4 $^{b, A}$
Group V	44±2.4 $^{b, A}$	125.3±11.4 $^{a, B}$	51.3±5.5 $^{b, A}$

Each value represents mean ±SE

Differently letters small or capital indicated there is significant difference (p≤0.05). Horizontal Significant difference represented by capital superscripted and vertical Significant difference represent by small superscripted. Time represent:0 days:normal diet(negative Control);60days:2% cholesterol diet for all groups except groupI(Control positive);74 days:treatment with partial pure ,pure lignan and galantamine diet for 14 days after 60days of cholesterol diet according to groups treatment program, …..=scarified animal.

The effect of flax lignan on high density lipoprotein (HDL) level:

Table 3. show the result of mean value of serum HDL level (mg/dl) before and after treatment . Cholesterol-treatment (group II) have shown a significant (P≤0.05) increase in mean value of serum HDL level(209.7 mg/dl) compared to negative control group (group I) amount of (43.8 mg/dl).Partial pure lignan -treatment(group III) have shown a significant (P≤0.05) decrease in mean value of serum HDL level(30.5mg/dl)compared to positive control

group(groupIII)amount of (132.3 mg/dl). Pure lignan-treatment (group IV) have shown a significant (P≤0.05) decrease in mean value of serum HDL level (37.5 mg/dl) compared to positive control group(group IV)amount of (255.8 mg/dl). Galantamine-treatment(group V) have shown a significant (P≤0.05) decrease in mean value of serum HDL level(48 mg/dl)compared to compared to positive control group(group V)amount of (397.5 mg/dl). The results from this study clearly demonstrated that 60 days of 2% of high cholesterol diet feeding significantly increased serum total cholesterol, LDL, HDL and TG, These results agree with other studies, Mohammadi *et al.*,(2006) who reported that (cholesterol ,HDL and LDL)level were significantly increased when (2%) high-cholesterol fed rabbits for 120days.However, an unexpected outcome in our study was the increase in HDL in treated rabbits after 60 days (Group III, Group IV, Group V), but this is consistent with some previous studies which found that serum HDL level increased when high-cholesterol fed rabbits for 120days (Zatta *et al.*, 2002). The decrease in mean value of serum HDL level of both lignan compounds in treated rabbits have been agreed with many studies. In animal models of AD, there is a report of a reduction in HDLs in mice (Li *et al.*, 2006) and in AD patients treated with atorvastatin (Murphy *et al.*,2010)

Table 3. Effect of lignan compounds and galantamine on mean serum high density lipoprotein (HDL) (mg/dl) level after treatment.

Experimental Groups	Treatment periods		
	0 days	60days	72 days
Group I	43.8±2.2 $^{a, A}$	43.8±2.2 $^{a, D}$	43.8±2.2 $^{a, A}$
Group II	43.5±1.2 b, ^{A}	209.7±63.6 $^{a, B}$	……
Group III	41.8±1.2 $^{b, A}$	132.3±21.9 $^{a, C}$	30.5±1.6 $^{b, A}$
Group IV	36.8±1.1 $^{b, A}$	255.8±80.2 $^{a, B}$	37.5±4.4 $^{b, A}$
Group V	34.5±2 $^{b, A}$	397.5±35.1 $^{a, A}$	48±3.2 $^{b, A}$

Each value represents mean ±SE

Differently letters small or capital indicated there is significant difference (p≤0.05). Horizontal Significant difference represented by capital superscripted and vertical Significant difference represent by small superscripted. Time represent:0 days :normal diet(negative Control);60days:2% cholesterol diet for all groups except groupI(Control positive);74 days:treatment with partial pure ,pure lignan and galantamine diet for 14 days after 60days of cholesterol diet according to groups treatment program, …..=scarified animal.

Table 4. Show the result of mean value of serum LDL level (mg/dl) before and after treatment. Cholesterol-treatment group (positive control group, group II) have shown a significant (P≤0.05) increase in mean value of serum LDL level (930.5 mg/dl)compared to negative control group(group I) amount of (3 mg/dl). Rabbits treated with 40mg/kg of Partial pure lignan (group III) show a significant (P≤0.05) decrease in the mean value of serum LDL level (103 mg/dl) as compared to positive control group (groupIII) amount of (665 mg/dl) but significant (P≤0.05) less than pure lignan-treated group. Rabbits treated with 40mg/kg of pure lignan (group IV) show a significant (P≤0.05) decrease in the mean value of serum LDL level(3.3 mg/dl)as compared to positive control(group IV) amount of (780.3 mg/dl) but significant (P≤0.05) much more than partial pure lignan-treated group . Galantamine-treatment (group V) have shown a significant (P≤0.05) decrease in mean value of serum LDL level (10mg/dl) compared to positive control (group V) amount of (655 mg/dl). These results of increase in LDL have been also agreed with Roberts *et al.* (2002) who observed that higher saturated and monounsaturated fat caused an increase in plasma triglycerides, total-cholesterol, VLDL and LDL. Cholesterol feeding in rabbits caused a significant increase in the circulating total cholesterol, LDL-cholesterol, VLDLcholesterol. These results are consistent with earlier report (Jain *et al.*, 2007) and Devasagayam *et al.*, (2004) suggested that oxidative modification of low-density lipoproteins (LDL) caused by reactive oxygen species result in the formation of foam cells which is the initial lesion of atherosclerosis.

Table 4. Effect of lignan compounds and galantamine on serum low density lipoprotein (LDL) (mg/dl) level after treatment.

Experimental Groups	Treatment periods		
	0 days	60days	74 days
Group I	3±0.4 ^{a,A}	3±0.4 $^{a, D}$	3±0.4 $^{b, B}$
Group II	3.3±0.8 ^{b,A}	930.5±71.1 $^{a, A}$	…….
Group III	3.5±0.6 ^{c,A}	665±57.4 $^{a, C}$	103±13.2 ^{b,A}
Group IV	4.8±0.9 $^{b, A}$	780.3±62.3 $^{a, B}$	3.3±0.5 $^{b, B}$
Group V	4.5±1.2 $^{b, A}$	655±90.4 $^{a, C}$	10±1.5 $^{b, B}$

Each value represents mean ±SE

Differently letters small or capital indicated there is significant difference ($p \leq 0.05$). Horizontal Significant difference represented by capital superscripted and vertical Significant difference represent by small superscripted. Time represent:0 days:normal diet(negative Control);60days:2% cholesterol diet for all groups except groupI(Control positive);74 days:treatment with partial pure ,pure lignan and galantamine diet for 14 days after 60days of cholesterol diet according to groups treatment program, …..=scarified animal.

They also reported that LDL oxidation and atherosclerosis can be inhibited by nutritional antioxidants. There are also epidemiological evidences and interventional studies to correlated higher level of antioxidant-rich food uptake with lower incidence of coronary heart disease. In the present study, rabbits males an increase was found in LDL levels in High cholestrol diets. In this way, long-term HF diet can contribute to the elevation of serum VLDL and TG.These results concide with Prasad, (2009) who suggests that high cholesterol diet (0.25%) for 120 days caused an increase in the serum level of TG, TC, LDL.

The decreases in the serum TC and LDL with partial lignan could be due to the SEG content of the Partial lignan (Van Niekerk *et al.*, 1984). SEG is a phytoestrogen known to reduce serum TG, TC and LDL (Arjmandi *et al.*, 1998). One of the several possible mechanisms that have been proposed for hypocholesterolemic effects of lignans is that an increase in bile acid excretion induced by flaxseed ingestion enhances the removal of LDL and alters hepatic metabolism in a way that augments LDL removal by hepatocytes, and increases LDL receptors (Lissin and Cooke, 2000). The other striking finding in the Newairy and Abdou, (2009) study is that flax lignans nearly normalized the lipid profiles in serum and liver of rabbit, and become near normal values of controls.

The oxygen radical scavenging properties of FS lignans were shown *in vitro* by either direct hydroxyl radical scavenging activity or inhibiting lipid peroxidation (Newairy and Abdou, 2009). The antioxidant properties of FS lignans were also found in models of endotoxic shock in dogs (Prasad, 2000).The flaxseed lignan SEG and its metabolites (ED and EL) have known antioxidant activities, shown both *in vitro* (Pattanaik and Prasad, 1998) and *in vivo* systems (Kitts *et al.*,1999), that are exerted mainly through the inhibition of lipid peroxidation.The phenolic lignans and other phytoestrogens have antioxidant activity (Kitts *et al.*, 1999). We supposed that the hypolipidemic effect of flax lignan could be due to a multi mechanisms that are reported in several studies, Flax Lignan(FL) may increase the protein levels of 7-α hydroxylase, which is involved in the conversion of cholesterol to bile,(Lucas *et al.*, 2004). Zanwar *et al.*, (2011) showed that FL which contains SEG lignan, is biphenolic and an effective scavenger of reactive oxygen species and can inhibit lipid peroxidation through chelation of transition metal ions or their chain-breaking antioxidant activity. From these results it can be concluded that flaxseed contains active components (lignans) which decrease serum lipid profile and lowers the risk of atherosclerosis in cholesterol fed rabbits may be through the inhibition of lipid peroxidation and through antioxidant activity.

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