

Protective role of soybean lecithin in reducing hypercholesterolemia and DNA fragmentation inducing by high cholesterol in adult male rats

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Abstract

The present experiment was investigated to study the ameliorative role of soybean lecithin specially phosphatidylcholin on some lipid profiles, glycemic index and DNA damage in intact and hypercholesterolemic infected rats. Thirty two adult male rats have been used in this study, were randomly selected and equally divided in to four groups as follows C, T1, T2, T3. They were treated orally (daily) for 42 days as follows; C:control group, were given distilled water by gavage needle, rats of this group were given soybean lecithin only (430mg\kg\day) orally; T2:rats of this groups were given only cholesterol (10gm\day) orally; T3: rats of this groups were given soybean lecithin (430mg\kg\day) orally, and high cholesterol (10gm\day) orally. The daily supplementation of soybean lecithin induces a significant decrease (p < 0.05) in total cholesterol (TC) and triglyceride (TG) in both intact and hypercholesterolemic infected rats respectively. Moreover, the glycemic index reveals a significant decrease in both glucose concentration and insulin resistance, whereas elevation in the concentration of insulin hormone significantly was noticed.In T3 group the results of Agarose Gel Electrophoresis showed that animals given Cholesterol plus soybean lecithin showing improvement in DNA damage by decreasing the fragmentation and increasing the condensation and concentration. In conclusion, the results from this experiment confirm that soybean lecithin supplementation to rats has an important protective role on cardiovascular system and liver in hypercholesterolemic infected rats. This supplementation can overcome the deleterious effect of hypercholesterolemia on heart and liver basically.

Keywords: Soybean lecithin, Cholesterol, Hypercholesterolemia, Insulin resistance

تم اختبار التجربة الحالية لدراسة الدور التحسيني للـ لسيثين فول الصويا خاصة الفوسفاتيديل كولين على بعض المعابير الدهنية ، ومؤشر نسبة السكر في الدم ، وتلف الحمض النووي في الجرذان المصابة بفرط كولستيرول الدم. تم استخدام اثنان وثلاثين من ذكور الجرذان البالغة في هذه الدراسة ، تم اختيارهم عشوائياً ومقسمة بالتساوي في أربع مجموعات على النحو التالي C، T1، T3، T7، T3, The (T2 كانت تعامل فمويا (يوميا) لمدة 42 يوما على النحو التالي ؟ C: مجموعة السيطرة ، أعطيت الماء المقطر بواسطة انبوب التجريع ، أعطيت الجرذان من هذه المجموعة الليسيثين فول الصويا فقط (409430 \ mg) فمويا T2: أعطيت الجرذان من هذه المجموعات فقط الكولسترول (my \ kg \ day430) فمويا T3: أعطيت الجرذان من هذه المجموعات فقط الكولسترول (mg \ kg \ day10) فمويا T3: أعطيت الجرذان من هذه المجموعات لقط الكولسترول (mg \ kg \ day400) فمويا T3: أعطيت الجرذان من هذه المجموعات الليسيثين فول الصويا (mg \ kg \ day430) فمويا عن طريق (mg \ kg \ day430) فمويا T3: أعطيت الجرذان من هذه المجموعات الليسيثين فول الصويا (mg \ kg \ day430) فمويا ، وارتفاع نسبة الكوليسترول (mg \ kg \ day40) عن طريق الفم. المكملات اليومية من الليسيثين فول الصويا يدفع انخفاض كبير (0.0 <P) في إجمالي الكوليسترول (TC) و الدهون الثلاثية (TG) في إحمالي الكوليسترول (TC) و الدهون الفم. المكملات اليومية من الليسيثين فول الصويا يدفع انخفاض كبير (0.0 <P) في إجمالي الكوليسترول (TC) و الدهون الثلاثية (TG) في إحمالي الكوليسترول (TC) و الدهون الفم. المكملات اليومية من الليسيثين فول الصويا يدفع انخفاض كبير (0.0 <P) في إحمالي الكوليسترول (TC) و الدهون الثلاثية الترول (TC) في إحمالي الكوليسترول (TC) و الدهون الفم. المكملات اليومية من الليسيثين فول الصويا يدفع انخفاض كبير (0.0 <P) في إحمالي الكوليسترول (TC) و الدهون الثلاثية التراثية الترول (TC) في إحمالي الكوليسترول (TC) و الدهون الثلاثية التراثية (TC) في إحمالي الكوليسترول (TC) و الدهون الثلاثية التراثية على التراثية على إلى التراثين في إحمالي الكوليسترول (TC) و الدهون الثلاثية التراثين أرح (TC) في إحمالي الكوليسترول (TC) و الدهون التراثية على الثلاثية مؤمل الحمول (TC) في كل من الحران في والعالي وعلين مول الصويا الحرون (TC) و الدون الحرون (TC) في إحمالي الكوليسترول (TC) في كل من تركيز في ألهون ألي في إلى أرح ألى ألكولي ألمومين في ل ألمومي في ألمومي في ألمومي في ألمومي في أ محوط في كل من تركيز الجلوكوز ومقاومة الأنسولين ، في حين لوحظ ارتفاع في تركيز هرمون الألاسولين بشكل أليسيتي في أل اليسيثين فول الصويا تظهر تحسنا في تلف الحمض النووي عن طريق تقليل التحطم وزيادة الكثلة والترموية توكي في في ألموي في في أ

1.Introduction

Hypercholesterolemia is а lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol. It has been reported by Rerkasem^[1] as one of the most important risk factors in the development and progression of atherosclerosis that lead to cardiovascular diseases (CVDs). Hypercholesterolemia poses a major problem to many societies as well as health professionals because of the close correlation between cardiovascular diseases and lipid abnormalities ^{[2] [3]}. Dietary factors such as continuous ingestion of high amounts of saturated fats and cholesterol are believed to be directly related to hypercholesterolemia and susceptibility to atherosclerosis ^[4] s.Hypercholesterolemic animals are useful models for studies on cholesterol homeostasis, and drug trials to better understand the relationship between disorders cholesterol metabolism. in atherogenesis as well as possible treatments for the reduction of circulatory cholesterol levels. Inducing hypercholesterolemia in rats is often through a high fat, high cholesterol diet, with the fat source varying from lard to canola, coconut, palm oil. Commercial

rations supplemented with cholesterol have also been used for these investigations ^[5]

Soybean is a legume that have no cholesterol and is less in saturated fat. Soybeans have been expended as a major export of protein by people in Asia for centuries, while their expenditure in the West only spans several decades. The soybean is the only vegetable food that consists of all eight essential amino acids. In 2009. the world soybean manufacture was 210.9 million metric tons, about 53% of the world oilseed production.Lecithin enriched diet can customize the cholesterol homeostasis and lipoprotein metabolism. Lecithin diet modifies the cholesterol homeostasis in the liver, increasing the activity of HMG-CoA reductase and cholesterol alpha-7 hydroxylase, and decreasing the microsomal ACAT activity^[6].

Insulin resistance develops as а consequence of the effects of inflammatory endoplasmic hormonal factors. and reticulum (ER) stress, and accumulation of by-products of nutritional 'overload' in insulin-sensing tissues. Although several of the damaging mechanisms are common across organs and tissues, others may be which more specific. highlights the significant challenges in designing intervention pharmacological for this ^[7]. Simultaneously, condition in both

animals and humans, the triggering factor for the transition from an obese, insulinresistant state to full blown type 2 diabetes is β -cell failure, which involves both a partial loss of β -cell mass and a deterioration of β -cell function. Some of the mechanisms that are involved in β -cell failure are similar to the mechanisms of insulin resistance ^{[8].}

In accordance with this, the recent study was focused to investigate the possible treatment role of soybean lecithin to hypercholesterolemia induced by high cholesterol in adult male rats.

2. Materials and methods 2.1 Experimental animals and care

Healthy local Thirty two male Albino Wistar rats their ages between (3 -4) months old and there weights between 190 -200 gram (g) were obtained from the drug control center /ministry of health and reared in the animals house of the Veterinary Medicine College /Green AL.Qassim University during the period extended from February to April,2017. They were reared in suitable condition of 20-25 °C in an air conditioned room and photoperiod of 12 hours daily. The animals were housed at least two weeks for acclimatization before beginning the experiment.Anticoccidiosis (Amprolium) was given via drinking water (1g/litter) for three days during acclimatization period.

2.2 Preparation of soybean lecithin:

After the pilot study ,One softgel capsule of soybean lecithin (1200gm) was dissolved in in 4ml olive oil orally administered to male rats at a dose of (150mg/rat/day) by using gastric intubation ^[9].

2.3 Preparation of cholesterol:

Hypercholesterolemic diets were supplemented with 10 g of powder cholesterol per kg of diet ^[10].

2.4 Blood samples collection

At the end of the (42 day) of the experiment, fasting blood was obtained via cardiac puncture from each rat. Samples were divided into two divisions, the first part is collected by anticoagulant tube for DNA damage technique, and the second part for serum collection and was isolated after centrifugation at a speed of 3000 revolution/minute (rpm) for 20 minutes. Serum samples were stored in a freezer at -18 °C until use^[11].

2.5 Experimental design for the experiment

After acclimatization for two weeks the rats were divided equally into four groups, (C) control group which received distilled water daily, (T1) rats of this group were given *soybean lecithin* only (430mg\kg\day) orally; (T2) rats of this group were given only cholesterol (10gm\day) orally; (T3) rats of this group were given *soybean lecithin* (430mg\kg\day) plus high cholesterol (10gm\day) orally.

2.7 Statistical analysis

The statistical analysis of the data of the experiment was performed by using oneway ANOVA and Least significant differences (LSD) to assess significant differences among means of the groups by using the SAS^{[12].}

3. Results and Discussion

3.1 Adverse impact of cholesterol on some lipid profiles and glucose concentration , insulin hormone, insulin resistance :

Table (1) indicated that mean values of serum total cholesterol and serum Triglyceride concentration after (42) days of experiment significantly (P<0.05) decreased after oral intubation of soybean lecithin (T3) comparing to (T2) and control group, this indicating hypocholesterolemic effect of soybean lecithin. The results also showed that HDL-Cholesterol increased significantly (P<0.05) in soybean lecithin plus cholesterol group (T3) comparing to the cholesterol

group (T3) and control group. At the end of the experiment, the mean values of this parameter were $(73.37\pm1.25b)$, $(60.00\pm0.46b)$ and $(36.37\pm0.86a)$ for(T3) respectively comparing with (T2) and control group.

The effect of oral intubation of soybean lecithin (T3) on mean value of some glycemic index are clarified in table (2).Depending on statistical analysis there were significant (P>0.05) decreased in glucose concentration and insulin resistance (T3) group with mean of values (99.37±1.38b) and (4.34±0.14b) respectively as compared with (T2) (128.75±1.95a) and $(7.25\pm0.29a)$ and control group, while the concentration of insulin hormone was elevated significantly (P>0.05) (18.06±0.06b) in (T3) as compared with (T2) which it is with mean value (12.48±0.38c).

Result of current study revealed that the oral admisteration of pure cholesterol to male rats causes significant increase in serum Total cholesterol, serum LDL Cholesterol, and triglyceride but it decrease in serum HDL cholesterol in hypercholesterolemic treated rats group.The results reported here in conferred with the results of ^[13], which noted that feeding rats a diet rich in cholesterol evoked an increase in risk factors for atherosclerosis and CVD such as dyslipidemia (high TG,total and LDL cholesterol .and low HDL Cholesterol).It also stimulated food intake with a concomitant increase in body weight gain. Also, another study indicate that high fat and cholesterol diet induced elevated level of total serum cholesterol ^{[14].}A study showed that а high fat/cholesterol diet did not induce changes in body weight, because this type of diet induces a decrease in testosterone, which is crucial in muscle bulding, and fat deposition [15]

The results revealed that there is a significant decrease in insulin hormone concentration at day 42 of the experiment in hypercholesterolemic treated rats group. Insulin is one of the most important hormones in the regulation of nutrient metabolism. In lipid metabolism, insulin enhances lipoprotein lipase activity ^[16], and fatty acid synthesis and inhibits lipolysis ^[17] Insulin mediate the plasma lipid profiles plasma concentrations like the of choleasterol esters and phospholipids so by that insulin increase low-density lipoprotein receptor expression [18] and the activity of LDL uptake into the cells ^[19]. Therefore, feeding on high cholesterol diet enhance the removal of cholesterol esters and phospholipids circulation.In from our present study high level of glucose is observed by action of high cholesterol.Hyperglycemia in cholesterol group could be explained by the higher metabolic energy derived from fat, and the level of glucose elevated slightly at the end of experiment. High fat, high energy diet alter intestinal paracellular permeability leading to increase in lipopolysaccharide (LPS) level in plasma, and this is related to the change in gut microbiota. This study refers that LPS plays a significant role in glucose modulating intolerance and atherosclerosis ^[20].

Intubation of 10g/day pure cholesterol in rats caused a significant increase in insulin resistance.Insulin regulates many aspects of lipoprotein metabolism. Resistance to the normal actions of insulin causes the hepatic overproduction of TG and apoB, which thereby enhances the secretion of very lowdensity lipoproteins from the liver ^[21].In addition, IR decreases lipoprotein lipase activity, resulting in a delayed clearance of TG-rich lipoproteins. It is generally believed that a delayed clearance of TG-rich lipoprotein is associated with the generation small of dense LDL and lower

concentrations of HDL-C ^{[22].}IR was significantly correlated with TG, apoB, HDL-C, and LDL size. Taken together, these findings suggest that IR may play an important role in lipid especially metabolism.Adiposity, the accumulation of visceral fat, increases intraportal free fatty acid (FFA) levels and flux, thereby inhibiting insulin clearance and promoting IR^[23]. In addition, an increased the secretion or decreased in of adipocytokines form adipocytes, such as leptin, tumor necrosis factor (TNF)- α , adiponectin may cause IR ^{[24].}The insulin resistance is positively correlated with TG and LDL-c levels but negatively correlated with HDL-c levels ^{[25].}

3.2 Protective effect of soybean lecithin on some lipid profiles and glucose concentration , insulin hormone, insulin resistance :

Dietary phosphatidylcholines explored for their ability to reduce plasma cholesterol and hence contributing significantly to cardio protective property. Soybean is a dietary phospholipids advocated for use in lowering serum total cholesterol levels in patients with

hypercholesterolemia.Phosphatidylcholine

(lecithin) may be able to reduce serum lipids by increasing bile acid excretion .Several studies suggest that a lecithin enriched diet can modify the cholesterol homeostasis and lipoprotein metabolism.Lecithin diet modifies cholesterol homeostasis in the liver by increasing the activity of HMG-COA reductase and cholesterol 7 alphahydroxylase, and decreasing the microsomal ACAT activity^[26].

One of the most spectacular properties of lecithin is its ability to reduce the excess of LDL cholesterol.It also promotes the synthesis in the liver of great amount of HDL, the beneficial cholesterol ^[27]·Bile acid secretion with high level of cholesterol and phospholipids is encouraged by lecithin-rich diets when compared with diets without lecithin Therefore,this study evaluates hypercholesterolemic effect of soy lecithin on patients with pure or combined hypercholesterolemia.In study on broilers fed soy-lecithin it has lower serum total cholesterol and triglyceride and had the highest insulin level.The result of this study impliedthat soy-lecithin with soy-oil had highest growth performance and soylecithin had cholesterol lowering capacity^[28]

This study establishes that feeding rats diet enriched in soybean lecithin results in remarkable changes in hepatic cholesterol homeostasis. The major finding is a decrease of ACAT activity and an altered cholesterol compartmentalization in the liver. Previous work indicated that this enzyme plays a pivotal role in cholesterol distribution in the hepatocyte, specifically, whether it is diverted to lipoprotein or biliary pathways or to the cholesterol ester storage compartment ^[29].In summary, dietary lecithin increases bile acid pool and stimulates biliary lipid secretion and bile which is associated with significant changes in hepatic cholesterol compartmentalization favoring a greater mobilization of cholesterol to bile. Diet intervention also drastically reduced ACAT activity and plasma VLDL as well as their triglyceride and cholesteryl ester content. Thus, lecithin feeding mimics the effect of drug-induced inhibition of ACAT activity which corroborates potential the antiatherogenic properties of lecithin supplementation ^[30]. There is no significant differences in body weight of T3 group as compared with the control group.

The results of our present study refers that glucose concentration significantly decreased by the daily intubation of soybean lecithin to male rats in T3 group as compared with T2 group, while insulin hormone increased in the same group, for

this reason the consumption of soybean lecithin with its isoflavones increases insulin secretion following a glucose challenge. Despite the increased insulin secretion there no were no changes in glucose disposal and a dose-dependent increase in the ratio of insulin: glucose AUCs determined from the IVGTTs, indicating an increase in peripheral insulin resistance due to the isoflavones. Further, consistent with an insulin resistance state there is a significant decrease in plasma adiponectin concentrations with sov isoflavones ^[31]. It was concluded that the metabolic responses of cod and soy proteins, when compared to casein, improved fasting glucose tolerance, and peripheral insulin sensitivity ^[32]. There is no significant differences in the concentration of IGF1 in soybean lecithin group and soybean lecithin plus high cholesterol group as compared with control group, as well as insulin resistance.

3.2 Effect of cholesterol and soybean lecithin on DNA damage:

The results obtained in the present show that hypercholesterolemia can promote DNA damage The influence of hypercholesterolemia on DNA damage has recently been observed in rats ^[33], including reduced oxidative DNA during dietary lipid lowering [34]. Additionally, we might also consider the relevance of ROS in this as recent studies show process. that cholesterol oxidation through ROS results in the formation of hydroperoxides, which might induce modifications and mutations in DNA ^[35]·Hypercholesterolemia results in an excessive production of ROS, which promotes oxidative stress and leads to cell damage and dysfunction ^[36]. Elevated levels of ROS can damage different types of molecules, including nucleic acids .Because soybean lecithin considered as antioxidant, so that ROS appear to play an important role in the generation of WBC DNA damage. Although in vitro studies have demonstrated a beneficial effect of antioxidant supplements in protecting sperm DNA from exogenous oxidants, the effect of these antioxidants in protecting WBC from endogenous ROS. The data suggest that dietary antioxidants may be beneficial in reducing WBC DNA damage, particularly, in men with high levels of DNA fragmentation. However, the mechanism of action of dietary antioxidants has not been established and most of the clinical studies are small ^[38].

Groups	С	T1	T2	T3	
	Intact Rats	Rats received	Rats received	Rats received	LSD
	Received	soybean lecithin	pure	soybean	
	distilled water	only	cnolesterol	lecitnin+pure	
			only	cnolesterol	
Parameters					
	68.75	67.12	237.25	73.37	
Total cholesterol	<u>±</u>	±	\pm	±	
(mg/dl)	1.54	1.21	6.44	1.25	8.8733
	В	В	А	В	
HDL-	35.37	33.87	12.12	36.37	
Cholesterol	<u>±</u>	±	<u>±</u>	±	5 99
(mg/dl)	0.82	0.71	0.29	0.86	5.00
	А	А	В	А	
Triglyceride	62.50	56.75	169.12	60.00	
(mg/dl)	±	±	\pm	±	
	4.66	1.61	1.94	0.46	6.881
	В	В	A	В	

 Table 1 Effect of cholesterol and soybean lecithin on some lipid profiles:

Values represent mean \pm SE (N=5).

Different capital letters denote a significant difference between groups ($p \le 0.05$).

Table2 The effect of cholesterol and soybean lecithin on Glucose concentration, insulin hormone, and insulin resistance:

Groups	С	T1	T2	T3	
	Intact Rats Received distilled water	Rats received soybean lecithin only	Rats received pure cholesterol only	Rats received soybean lecithin+pure cholesterol	LSD
Parameters	04.60	00.40	100 55	00.25	
Glucose (mg/dl)	94.62 ± 0.98 C	90.48 ± 0.66 D	128.75 ± 1.95 A	99.37 ± 1.38 B	3.6581
Insulin (g)	18.86 ± 0.28 AB	20.02 ± 0.35 A	12.48 ± 0.38 C	18.06 ± 0.06 B	1.250
Insulin Resistance(g)	4.40 ± 0.05 B	4.49 ± 0.07 B	7.25 ± 0.29 A	4.34 ± 0.14 B	0.451

Values represent mean \pm SE (N=5).

Different capital letters denote a significant difference between groups ($p \le 0.05$).



Fig 1 : DNA Damage Analysis (Agarose Gel Electrophoresis).

Digital printout of an agarose gel electrophoresis Showing line(1):DNA from control WBC line(2) WBC DNA from 10g/kg.B.W. pure cholesterol(3) WBC DNA from 430mg/kg.B.W pluse 10g/kg.B.W. pure cholesterol group(4) WBC DNA from 430mg/kg/B.W. Lecithin treated group,line.

4. Conclusion

This work suggests that soy lecithin-rich diets can be used as an adjunct in the treatment of hypercholesterolemia. Lecithinrich diets can stimulate the fatty acid secretion with high levels of cholesterol and phospholipids when compared with diets without lecithin, considering the lecithin performance as phytotherapic, with a large spectrum of activity. The results showed significant reduction in the concentration of total cholesterol, suggesting that the daily administration of lecithin could be used as adiuvant treatment an in hypercholesterolemia, possibly by reducing the intestinal absorption or by the increased secretion of bile acids with high levels of cholesterol and phospholipids, and this will protect the body against CVD and Liver disease.

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