Original Research Article

Effect of Genistein on Lipid Profile and Oxidative Status in Hyperlipidemic Wistar Rats

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Abstract

Background: Hyperlipidemia is a major cause of atherosclerosis-induced conditions such as coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease. Due to various adverse effects with the current pharmacological therapy, many plant-derived compounds are being tested to lower serum lipid levels. Genistein, a polyphenolic isoflavone, showed promising results in several studies. **Objective:** The current study aimed to assess the effect of genistein on lipid profile and oxidative status in hyperlipidemic wistar rats.**Materials and Methods:** This prospective, observational *in vivo* study was carried out in Sri manakula vinayagar medical college and hospital, Puducherry, India. Thirty-xix adult male albino wistar rats were used for the experiment. Rats were divided randomly into six groups consisting of six animals in each group. All animals were given high cholesterol diet (0.75% cholesterol + 1.5% bile salt) to induce hyperlipidemia. The animals were treated with atorvastatin (10 mg/kg oral) and genistein (1 mg/kg oral and 5 mg/kg oral) once daily for a period of 30 days. All animals were weighed at the start and the end of the experimental period.On 31st day after overnight fasting, blood samples were collected from orbital sinus for biochemical analysis of lipoproteins and antioxidants. Basic parameters like body weight was measured. Measurement of parameters like Total Cholesterol, HDL, VLDL, LDL Triglycerides was carried out for undertaking Serum Lipid Profile. And, measurement of parameters like reduced glutathione (GSH), serum malondialdehyde (MDA) and plasma catalase was carried out for undertaking Antioxidant activity. Statistical analysis was performed by one-way analysis of variance test followed by post hoc Dunnett's multiple comparison test. $p<0.05$ was considered statistically significant.**Result:** Oral administration of genistein showed a significant reduction in body weight serum total cholesterol, triglycerides, and low-density lipoprotein levels. Dose dependent significant changes ($P<0.05$) were observed in MDA and Catalase levels among genistein treated animals. **Conclusion:** The current study demonstrated that genistein effectively attenuated raised serum TC, LDL, VLDL, and TG levels with remarkable improvement in antioxidant activity. Thus, genistein, a polyphenolic isoflavone, in a dose of 5 mg/kg alone and in combination with conventional hypolipidemic drug, atorvastatin has beneficial effect on serum lipid profile and antioxidant activity in hyperlipidemic male albino Wistar rat.

Keywords: Antioxidant, Genistein, Hyperlipidemia, Wistar rats

Introduction

In developing countries, cardiovascular disease (CVD) is one of the primary causes of mortality and disability [1]. In India alone, an estimated 31.8 million people suffer from coronary artery disease (CAD). India has three times the age-standardized estimates for disability-adjusted life years lost owing to CAD than developed countries [2].Elevations in total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and triglycerides (TGs), as well as low levels of high-density lipoprotein (HDL) cholesterol, are all symptoms of dyslipidemia [3]. Hyperlipidemia has a significant link to coronary artery disease, cerebrovascular stroke, and peripheral vascular disease. Hyperlipidemia can be caused by a main genetic flaw or a secondary cause such as a high-fat diet, medicines (isotretinoin and protease inhibitors), or illnesses (diabetes, nephrotic syndrome, hypothyroidism, etc.). Statins, fibrates, niacin derivatives, bile acid-binding resins, and cholesterol absorption inhibitors are all used to treat dyslipidemia. Chronic use of these medications has been linked to a number of side effects, including myopathy, hepatic dysfunction, gastrointestinal problems, and rashes [4].This necessitated the development of novel hypolipidemic medicines with fewer side effects. Genistein is a kind of isoflavone that may be found in large amounts in soybean extracts. It has gotten a lot of attention because of its estrogenic action, thus the name phytoestrogens. Genistein has been identified as a possible drug for the prevention and treatment of cancer and other chronic diseases such as osteoporosis, diabetes, postmenopausal syndrome, and cardiovascular disease due to its multidirectional effect on living cells [5]. Furthermore, cellular investigations have shown that soy isoflavones influence peroxisome proliferator activator receptor (PPAR)-directed gene expression and have a positive impact on lipid and glucose metabolism. One reason for genistein's cholesterol-lowering effect might be the inhibition of hepatic lipid production [6]. It has been stated that genistein can help prevent and cure illnesses including atherosclerosis, acute coronary syndromes, pulmonary hypertension, heart failure, and myocardial infarction by acting as an antiatherogenic agent [6]. In view of this, this prospective, observational was carried out to evaluate the effect of genistein on lipid profile and oxidative status in hyperlipidemic wistar rats in hyperlipidemic male albino Wistar rats.

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Method

Experimental Animals

Laboratory-bred adult male albino Wistar rats (10–12 weeks old) having body weight in the range of 180–250 g were purchased from Tamilnadu veterinary and animal sciences university, Madhavaram milk colony, Chennai, India. They were kept in the animal house

under controlled conditions of illumination (12h light/12h darkness) and temperature 20–25℃ (airconditioned room) for 1 week before and during the experiments. They were maintained on standard pellet diet and water ad libitum throughout the experimental period. All procedures in the study were reviewed and approved by the institutional animal ethical committee (14/17/10/2013 IAEC certificate number). The animals were taken care as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals. Experiment was conducted based on good laboratory practice.

Drugs and Chemicals

Standard pellet diet purchased from Pranav agro industries, India composed of hypercholesterolemic diet – 0.75% cholesterol + 1.5% bile salt; and atorvastatin, solvents – polyethylene glycol (PEG), dimethylsulfoxide (DMSO) (Name of Company), genistein (Name of Company), cholesterol reagent kit and triglyceride reagent kit were procured and HDL cholesterol reagent kit was procured from subra scientific, Puducherry India, were used.

Experimental Procedure

Thirty-six adult male albino Wistar rats were divided randomly into six groups consisting of six animals in each group. This study was conducted over a period of 30 days.

Grouping of Animals

1) Group 1 - Standard pellet diet + Vehicle (DMSO)
2) Group 2 - High cholesterol diet.
3) Group 3 - High cholesterol Diet + Atorvastatin10mg/kg .
4) Group 4 - High cholesterol Diet + Genistein 1mg/kg .
5) Group 5 - High cholesterol Diet +Genistein 5mg/kg .
6) Group 6 - High cholesterol Diet + Atorvastatin10mg/kg + Genistein 5mg/kg .

Genistein was dissolved in 0.5 mL of 30% dimethylsulfoxide (DMSO). Animals were given high cholesterol diet and drugs daily through oral route along with the standard pellet diet for a period of 30 days. All animals were weighed at the start and the end of the experimental period.On the 31st day after overnight fasting, blood samples were collected from orbital sinus for biochemical analysis of lipoproteins and antioxidants.

Parameters Measured

Parameter I: Body Weight

Parameter II: Serum Lipid Profile including Total Cholesterol, HDL, VLDL,LDL Triglycerides

Parameter III: Antioxidant activity profile including estimation of reduced glutathione (GSH), estimation of serum malondialdehyde (MDA) and estimation of Plasma catalase.

Statistical Analysis

Data were entered and analyzed using spss software version 16.0 by one way analysis of variance (anova) and results were expressed as mean ± standard deviation (sd). Significance of difference between groups was further analyzed with dunnett's test for post-hoc comparisons. P value of <0.05 was considered statistically significant.

Results

This prospective, observational *in vivo* study which was carried involved thirty-xix adult male albino wistar rats. The rats were divided randomly into six groups consisting of six animals in each group.

Body Weight

Table 1: Effect of Genistein, Atorvastatin and other experimental variables on Body Weight in Experimental Animals

Groups	Treatment (mg/kg, oral route of administration)	Body weight in grams
I	Standard pellet diet + Vehicle (DMSO)	152.92±5.67
II	High cholesterol diet	271.17±12.33
III	High cholesterol Diet + Atorvastatin10mg/kg	201.17±11.14
I	High cholesterol Diet + Genistein 1mg/kg	227.83±11.49
V	High cholesterol Diet +Genistein 5mg/kg	178.33±8.16
VI	High cholesterol Diet + Atorvastatin10mg/kg + Genistein 5mg/kg	165.56±9.77

Effect of Genistein, Atorvastatin and other experimental variables on Body Weight in Experimental Animals is maintained in above Table 1. On the expected lines, the maximum body weight (271.17±12.33 grams) and the minimum body weight (152.92±5.67 grams) was recorded for adult male albino wistar rats who were fed with only high cholesterol diet and Standard pellet diet + Vehicle (DMSO), respectively for 30 consecutive days. This was followed by adult male albino wistar rats who were fed with High cholesterol Diet + Genistein 1mg/kg for 30 consecutive days, who recorded body weight as 227.83±11.49 grams. Adult male albino wistar rats who were fed with High cholesterol Diet + Genistein 5mg/kg for 30 consecutive days, recorded body weight as 178.33±8.16 grams. Important observation noted from this table is the genistein-related dose-dependent decrease in body weight of adult male albino wistar rats. In spite of high cholesterol diet, the consecutive consumption of Genistein at higher levels for 30 days did not increase the body weight. The metabolism of genistein has some metabolic links with lipid pathways.

Effect of Genistein on Serum Lipid Profile in Experimental Animals

Table 2: Effect of genistein on serum lipid profile in experimental albino Wistar rats

Groups	Treatment (mg/kg, oral)	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)
I	Standard pellet diet + Vehicle (DMSO)	72.83±4.26	113.00±4.73	26.67±5.05	22.60±0.95	23.57±1.73
II	High cholesterol diet	151.17±9.66***	220.00±15.50***	44.83±4.58***	44.00±3.10***	62.33±3.80**
III	High cholesterol Diet + Atorvastatin10mg/kg	99.83±12.61**	140.00±9.63***	30.33±3.98*	28.00±1.93**	41.50±15.93***
IV	High cholesterol Diet + Genistein 1mg/kg	110.33 ±18.04*	133.83 ±12.12*	30.50 ±9.57*	26.77 ±2.42*	53.07±9.54*
V	High cholesterol Diet +Genistein 5mg/kg	98.17±15.98***	109.00±10.47**	30.50±6.22***	21.80±2.09**	45.87±21.93*
VI	High cholesterol Diet + Atorvastatin10mg/kg + Genistein 5mg/kg	81.17 ±11.62**	104.50 ±9.35***	41.67 ±5.68***	20.90 ±1.87***	18.60 ±8.06*

Values are expressed as mean±SD for all six groups (n=6 in each group). *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle, cholesterol group, atorvastatin group, 1 mg/kg genistein group, 5 mg/kg genistein group and Atorvastatin10mg/kg + Genistein 5mg/kg group. Comparison was done by one-way analysis of variance followed by post hoc Dunnett's test. LDL: Low-density lipoprotein. TC: Total cholesterol. TG: Triglyceride. HDL: High-density lipoprotein. DMSO: Dimethylsulfoxide. SD: Standard deviation

Effect of genistein on serum lipid profile in experimental albino Wistar rats is maintained in above Table 2. On the expected lines, the minimum values and the maximum values for Total Cholesterol, Triglycerides, HDL, VLDL and LDL in mg/dl was recorded for Group I (Standard pellet diet + Vehicle (DMSO) and Group II (High cholesterol diet), respectively. There was difference for values regarding Total Cholesterol, Triglycerides, HDL, VLDL and LDL in mg/dl between Group IV (High cholesterol Diet+Genistein 1mg/kg) and Group V (High cholesterol Diet+Genistein 5mg/kg). We recorded the genistein-related dose-dependent decrease in serum lipid profile of adult male albino wistar rats. Our study recorded the lowest values for all variables in serum lipid profile for Group VI (High cholesterol Diet+Atorvastatin10mg/kg+Genistein 5mg/kg).

Effect of Genistein on oxidative status in Experimental Animals

Table 3: Effect of genistein on oxidative status in experimental albino Wistar rats

Groups	Treatment (mg/kg, oral)	MDA	Catalase	GSH
I	Standard pellet diet + Vehicle (DMSO)	4.12 ±1.05	42.83±1.42	10.98±1.64
II	High cholesterol diet	6.54±0.89*	30.48±4.79 **	7.65±0.97 *
III	High cholesterol Diet + Atorvastatin10mg/kg	4.67±0.55$^{\#}$	37.60±2.75 $^{*\#}$	7.41±1.58 *
IV	High cholesterol Diet + Genistein 1mg/kg	5.16±0.17	32.03 ±3.68 **	8.83±2.31
V	High cholesterol Diet +Genistein 5mg/kg	4.25±0.29 $^{\#\#}$	39.23±0.66 $^{**\#£}$	12.25±1.23 $^{\#\#\#\ \$\$}$
VI	High cholesterol Diet + Atorvastatin10mg/kg + Genistein 5mg/kg	4.22±1.04 $^{£}$	41.90±2.96$^{£}$	11.32±2.78$^{£}$

Values are expressed as mean±SD for all six groups (n=6 in each group).
* P<0.05, ** P<0.01, *** P<0.001 as compared with vehicle groups.
$^{\#}$ P<0.05, $^{\#\#}$ P<0.01, $^{\#\#\#}$ P<0.001 as compared with high cholesterol group.
$^{\$\$}$ P<0.01 as compared with atorvastatin group.
$^{£}$ P<0.05 as compared with genistein 5mg/kg group.
MDA: malondialdehyde, GSH: Glutathione synthetase. Comparison was done by one-way analysis of variance followed by post hoc Dunnett's test. . DMSO: Dimethylsulfoxide. SD: Standard deviation

In vehicle treated animals, the mean value of serum Catalase, GSH and MDA were 42.83±1.41, 10.98±1.64 and 4.12±1.04 respectively. These values did not differ significantly between the vehicle treated animals.In the high cholesterol group, serum MDA levels (6.54±0.89) were increased significantly (P < 0.05), whereas Catalase (30.48±4.79, P < 0.01) and GSH levels (7.65±0.97, P < 0.05) were significantly decreased as compared to vehicle treated animals indicating the development of oxidative stress in high cholesterol treated animals.Atorvastatin (10 mg/kg, oral) treated animals showed a significant decrease in serum MDA levels (4.67±0.55) and increase in Catalase activity (37.60±2.75) as compared to high cholesterol treated animals. However, there was no significant change in GSH levels in atorvastatin treated animals.In Genistein (1mg/kg) treated group, no significant change in oxidative status were noticed in experimental animals. However, high dose genistein (5mg/kg) showed a significant decrease in serum MDA (4.25±0.29, P<0.01) levels and increase in serum catalase (39.23±0.66, P<0.05) and GSH levels (12.25±1.23, P<0.001) as compared to high cholesterol treated animals. Dose dependent significant changes (P<0.05) were observed in MDA and Catalase levels among genistein treated animals. Moreover, when compared with atorvastatin treated animals, high dose genistein (5mg/kg) treated animals showed a significant increase in GSH levels (P<0.01).

Discussion

In the current study, rats fed a high-cholesterol diet had significantly higher blood TC, TG, VLDL, and LDL levels than rats on a low-cholesterol diet. When compared to high cholesterol diet treated mice, genistein at two different dosages (1 and 5 mg/kg) substantially reduced blood TC, TG, VLDL, and LDL levels. TC and LDL levels in genistein-treated mice were equivalent to those in atorvastatin-treated animals, while TG and VLDL levels were considerably lower than in atorvastatin-treated animals.Yao et al. found that genistein-treated mice had significantly lower blood TC and TG levels than high cholesterol-treated animals in a research [7]. In another investigation, Kim et al. discovered that supplementing with genistein (4g/kg diet) lowered blood TC, LDL, and triacylglycerol levels in experimental mice [8]. The current study, on the other hand, contradicts Kim's findings that genistein had no effect on serum TC and TG in diabetic mice [9]. Reduced cholesterol synthesis and esterification; decreased cholesterol and bile acid absorption from the gastrointestinal tract; increased bile acid excretion; inhibition of hepatic glucose conversion to lipids; increased hepatic LDL receptor activity; and expression due to upregulation of hepatic catabolic genes (liver fatty acid catabolism genes), including sterol regulatory element binding protein 2 (SREBP-2) reg [10]. Transactivation of PPAR is linked to adipocyte differentiation, insulin sensitization, and adipogenesis and lipid storage in subcutaneous adipose tissue, resulting in visceral fat mass redistribution to subcutaneous tissue [11]. With varying doses, the specific action of genistein on liver X receptor (LXR)/SREBP-1c remains unclear. According to Kim et al., genistein (2 and 4 g/kg) reduced the expression of LXR-RXR-SREBP1-c genes and adiponectin gene activation. 8 Adipogenesis has been shown to be inhibited at low doses and enhanced at high concentrations by genistein [12].The antioxidant system is triggered by the accumulation of antioxidant molecules. However, studies demonstrating a significant improvement in the antioxidant system induced by genistein were comparable to our findings [7, 8]. One possible mechanism is upregulation of PPAR and reduced expression of TNF at both the pre- and post-translational levels [13]. Genistein also alleviates oxidative stress by modulating oestrogen receptors [14]. Based on previous studies, another possible explanation could be due to high plasma-free fatty acid-induced expression of liver. By reducing plasma-free fatty acids and decreasing CYP2E1 production, soy protein containing genistein may protect the liver against oxidative injury [15]. LDL oxidation is prevented by genistein, which also has a radical scavenging effect, activates antioxidant enzymes, and suppresses oxidative DNA damage [15].The study's merits were that it was conducted with two different genistein dosages. Furthermore, rats' hyperlipidemia was induced solely by diet, and drugs were administered orally as well, allowing the study to be extrapolated to humans in the future. However, the limitations include, the duration of the study was short and other properties of study was not explored.

Conclusion

In conclusion, the present study proved that genistein effectively attenuated raised serum TC, LDL, VLDL, and TG levels with remarkable improvement in antioxidant activity. Thus, genistein, a polyphenolic isoflavone, in a dose of 5 mg/kg alone and in combination with atorvastatin has beneficial effect on serum lipid profile and antioxidant activity in hyperlipidemic male albino Wistar rats.

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