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Assessment of Antihyperlipidemic Effect of Daidzein in High Fat and High Sugar Diet Induced Hyperlipidemia in Wistar Rats

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ABSTRACT

Obesity is a chronic disease, whose incidence is alarmingly growing, affecting not only adults but also children and adolescents. It is associated with severe metabolic abnormalities and increased cardiovascular morbidity and mortality. The present study was designed to evaluate the protective effect of Daidzein on high fat/high sucrose diet induced obesity and cardiovascular complications in rats. Obesity was induced by feeding high fat/high sucrose (HFSD) diet for 6 weeks. Male wistar rats were divided into control group, High fat and sugar diet group, and two treatment groups. The test drug daidzein was administered orally at a dose of 5mg/kg and 10mg/kg in the treated group for a period of last 4 weeks. At the end of study period body weight, food intake, glucose tolerance, lipid profile, antioxidants levels were assessed. Treatment with Daidzein in HFS diet fed rats significantly reduced oxidative stress, TG, cholesterol, LDL levels with a significant increase in HDL levels, had no effect on body weight gain and food intake. These results suggest that the daidzein decreased lipid levels and oxidative stress and also improved liver histoarchitecture in treated group compared to HFS diet induced obesity.

Keywords: Obesity, High fat and sugar diet, lipid profile, Daidzein and oxidative stress.

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INTRODUCTION

The leading cause of mortality worldwide, hyperlipidemia especially hypercholesterolemia is well established to be a major risk factor for the development of cardiovascular disease (CVD), and was recently revealed to be a contributing factor in rotator cuff injuries [1,2]. The most popular lipid-lowering drugs right now are statins, such as atorvastatin and lovastatin, because of how well they reduce plasma lipids. However, skeletal muscular damage, rhabdomyolysis, and hepatotoxicity are some of the undesirable side effects that have limited their use [3]. Finding and enhancing natural and efficient agents that can be helpful in controlling lipid metabolism is so crucial. Traditional Chinese Medicine has lately attracted increasing attention in the treatment of metabolic syndrome and started to be a frequent therapy for controlling symptoms in patients with hyperlipidemia [4,5].

A diet high in fat can lead to disorders similar to metabolic syndrome, which can have an impact on cardiovascular health Cholesterol [6]. Animal models are frequently used to induce obesity, insulin resistance, hypercholesterolemia, and atherosclerosis. High-fat diets, which include between 30 and 60 percent fat, are commonly utilized to produce these conditions. Animal models have been used to create or accelerate the development of atherosclerotic lesions through the use of a diet that is high in fat [7-11]. Additionally, this diet decreases the sensitivity of insulin receptors and the expression of GLUT4, which results in peripheral tissue that is resistant to insulin.

Legumes like soybeans contain daidzein, a phytoestrogen. Daidzein's chemical makeup resembles mammalian oestrogens, and it may replace or inhibit oestrogen and the oestrogen receptor (ER) complex. Thus, daidzein protects against many diseases, including diabetes, osteoporosis, breast cancer, and cardiovascular disease, which are associated to oestrogen control [12-14]. Daidzein also has ER-independent biological actions associated to its prospective anticancer effects, such as antioxidant, immunological regulator, and apoptosis regulation

Currently, there is a dearth of research into the effects of Daidzein on hypercholesterolemia in animal models. Hypercholesterolemia is the most prevalent metabolic disorder in humans. In light of the fact that a high-fat diet causes hypercholesterolemia in rats, this study aims to assess the effects of daidzein and the lipid-lowering medication atorvastatin on liver function tests, cholesterol levels, and early structural abnormalities of the liver tissue.

MATERIAL AND METHODS

Materials

High-Fat Diet Preparation

A high-fat diet was prepared according to the method previously described by Lassoued et al. [15], with some modifications. The high-fat diet was prepared from a mixture of a 78.9% standard mouse diet (normal diet), 1% cholesterol powder, 0.1% cholic acid, 15% corn starch, and 5% corn oil. Daidzein is obtained from Hetero pvt ltd, Hyderabad as gift sample. All the kits were purchased from sigma Aldrich.

Experimental Animals

Male Wistar rats weighing 200-220g were used and are procured from National Center for Lab Animal Sciences, National Institution of Nutrition, Hyderabad, India. They were housed in a group of six under environmentally controlled room with 12-h light/dark cycle and had free access to food and water. After seven days of acclimatization period, they were randomly selected for different experimental groups. All the experimental procedures were approved by the institutional animal ethical committee (IAEC) [I/IAEC/AU/16/2024/MWR♂, School of pharmacy, Anurag University].

Experimental animals are randomly divided into five groups of each six animals. Group- I is control in this, animals fed with regular diet and distilled water. Group- II is Disease control group, in this Animals fed with High fat and sugar diet (HFSD) for 6 weeks, Group- III was standard group (HFSD + Atorvastatin) in this animals fed with HFSD with standard drug (10mg/ kg). Group- IV and V Were Low and high dose treated (HFSD + Daidzein) in these animals fed with High fat and sugar diet for 6 weeks and drug (5mg/ kg and 10mg/kg) for last 2 weeks. During the experimental period, body weight and food intake were measured daily.

The treatments were given via oral for 14 days. The doses of Daidzein acid and Atorvastatin used in the present study were according to previous studies [8]. Blood samples were collected from the tail vein and used for serum lipid profile and antioxidant analysis. The rats were then sacrificed. The liver was dissected for histopathological analyses. The liver weight was measured at the end of the study period.

Biochemical Measurements of Serum Lipid Profile

A total of 700 µL of the blood sample was centrifuged at 6000 rpm (Eppendorf Centrifuge 5810R) for 20 min, and the resulting serum was used for lipid profile analyses. Serum total cholesterol (TC), TG, HDL, LDL, and VLDL were measured by using Erba Kits [16-18].

RESULTS

Blood glucose test

The effect of Daidzein powder on glucose tolerance test in fasted rats is shown in Table 1, Daidzein (10 mg/kg) significantly decreased blood glucose level in glucose fed rats at 90 minutes when compared with the control group. It also decreased the elevated blood glucose at 60 minutes after the glucose administration. Disease control group showed significant increase in blood glucose level when compared with the normal group. This data suggested that treatment with Daidzein showed better tolerance to exogenously administered glucose.

Table 1: Hypoglycemic activity of Daidzein

GROUP	GSH u/mg	SOD u/mg
Normal control	16.45±1.2	14.01±1.0
HSD	5.01±1.4$^{\alpha}$	6.08±1.3$^{\alpha}$
Atorvastatin	13.89±1.1^{a}	12.22±1.2^{a}
Daidzein Low	8.98±1.3$^{\alpha,a}$	13.24±1.4$^{\alpha,a}$
Daidzein High	12.01±1.2$^{\beta,a}$	11.89±1.2$^{\beta,a}$

Effect of drug on body weight

Body weight of animal were increased significant with high fat and sucrose diet in HFSD group compared with control group, upon treatment with daidzein and standard drug, body weight of animals was brought to normal when compared with HFSD group, this indicates increase in weight due to high fat and sucrose diet has been restored with normal body weight by daidzein (Table 2).

Table 2: Effect of drug on body weight

	Blood glucose level mg/dl			
GROUPS	0 min	30 min	60 min	90 min
Normal control	98.65± 5.53	86.91± 3.27	94.86± 2.84	93.61 ± 4.96
HFSD	266.77± 4.16$^{\alpha}$	281.02 ±6.18$^{\alpha}$	270.89±4.02$^{\alpha}$	259.19± 5.13$^{\alpha}$
Atorvastatin	277.46± 3.54	204.02 ± 4.37	157.73± 3.06	107.37± 4.05
Daidzein Low	272.02 ± 2.44$^{\alpha}$	219.67± 2.6$^{\alpha,a}$	193.95±5.23$^{\alpha,a}$	107.66± 3.87
Daidzein High	281.04± 1.22$^{\alpha,a}$	211.48±3.33$^{\alpha,a}$	177.03± 3.19$^{\alpha,a}$	108.14± 5.04

Effect of drug on lipid profile

The measured plasma HDL, LDL and VLDL levels in rats that consumed the daidzein-supplemented high cholesterol diet and Atorvastatin are shown in table 3. The difference between the plasma HDL, LDL and VLDL levels in Disease group and Normal control group was significant ($P < 0.001$). Plasma HDL level in treatment group was increased compared to disease control group where as LDL and VLDL levels in treatment group was reduced compared to HFSD group.

Table 3. Effect of drug on Lipid profile

GROUPS	Body weight before treatment gms	Body weight after treatment gms
Normal control	223±4.3	255±4.15
HFSD	239±2.4	362±2.26 α
Atorvastatin	230±6.33	248±.24a
Daidzein Low	231±5.34	258±5.25a
Daidzein High	236±3.31	248±3.3a

Effect of drug on Triglyceride and total Cholesterol

The measured plasma TG and TC levels in rats that consumed the daidzein-supplemented high cholesterol diet and Atorvastatin are shown in table 3 The difference between the plasma TG and TC levels in HFSD group and Normal control group was significant ($P < 0.001$).

Effect of drug on GSH and SOD

Antioxidant parameters like GSH and SOD of animals were decreased significantly with high sucrose diet in HFSD group compared with control group, upon treatment with test daidzein and standard drug SOD and GSH of animals were brought to normal when compared with HSD group this indicates decrease in GSH and SOD due to HSD had been restored to normal by Daidzein.

Table 4: Effect of drug on GSH and SOD

GROUP	HDL mg/dL	LDL mg/dL	VLDL mg/dL	TC mg/dL	TG mg/ dL
Normal control	39±1.3	26.1±1.2	13.8±1.3	56.23±1.3	63.35±1.2
HFSD	23.5±1.2 $^{\alpha}$	59.3±1.3 $^{\alpha}$	22.89±1.2 $^{\alpha}$	92.12±1.2$^{\alpha}$	103.43±1.4$^{\alpha}$
Atorvastatin	37.6±1.1^{a}	38.1±1.2$^{\gamma,a}$	16.1±1.2^{a}	63.03±1.4^{a}	66.54±1.0^{a}
Daidzein Low	35.89±1.3$^{\alpha,a}$	41.4±1.2 $^{\alpha,a}$	17.98±1.1$^{\alpha,a}$	69.0.1±1.1$^{\alpha,a}$	77.23±1.2$^{\alpha,a}$
Daidzein High	38.1±1.2$^{\alpha,a}$	37.1±1.3$^{\gamma,a}$	16.8±1.23$^{\gamma,a}$	65.03±1.0$^{\gamma,a}$	68.21±1.3$^{\gamma,a}$

Data presented as Mean ± SEM (n=6).

$^{\alpha}P<0.001$, $^{\beta}P<0.01$, $^{\gamma}P<0.05$ vs normal control NC

$^{a}P<0.001$, $^{b}P<0.01$ $^{c}P<0.05$ vs High Sugar diet HFSD

Histopathology of Liver

Figure 1 shows the histopathological findings of the liver tissue of a normal diet-fed rat (non-hypercholesterolemic), a high fat and sugar diet induced hypercholesterolemic rat, Daidzein and Atorvastatin, the tissue section of the normal group showed the normal liver architecture indicated by normal hepatic cells with the characteristic morphology of a well-preserved cytoplasm, a prominent nucleus, and sinusoidal spaces. However, the tissue section of the liver of the HFSD rats demonstrated severe architectural damage specified by hemorrhage, steatosis, and cholesterol deposition in the

cytoplasm. Treatment with daidzein and Atorvastatin reduced architectural damage and cholesterol deposition in the liver.

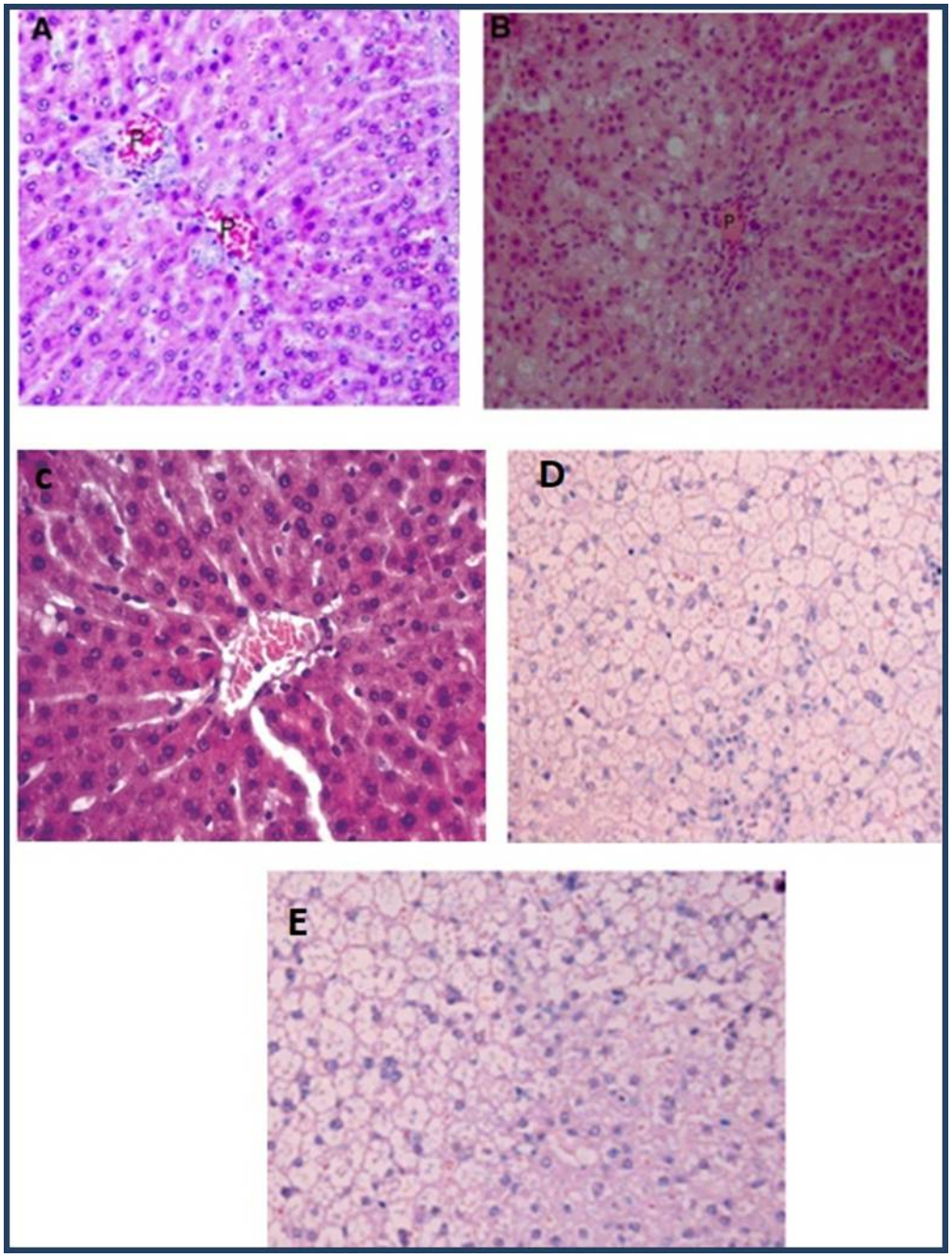

Figure 1. Effect of drug on Histopathology of liver such as A – Control, B- HFSD, C, D- Daidzein low and high dose E- Atorvastatin

DISCUSSION

A hyperlipidemic rat model was developed for this study by feeding Wistar rats a diet rich in saturated fats and cholesterol. This made it possible to investigate daidzein's potential hypolipidemic effects. Consistent with the results of a previous study, our results showed that rats given a high-fat diet clearly showed higher serum levels of triglycerides, total cholesterol, and low-density lipoprotein cholesterol [19-23]. When hyperlipidaemia is present, the body's cholesterol levels rise, which raises the level of lipid peroxidation and reactive oxygen species generation. One of the most significant risk factors for atherosclerosis, stroke, and heart attacks is arterial endothelial damage, which can be brought on by elevated blood levels of TC, TG, and LDL. Therefore, preserving the vascular endothelium and lowering total cholesterol levels must be given careful consideration in order to prevent cardiovascular disease. Lipid peroxidation in a high-cholesterol diet is generally caused by an excess of reactive oxygen species

and a decrease in the activities of antioxidant enzymes, which eventually results in the induction of pathological illnesses [24-27].

Consuming a diet high in fat has been shown to lead to a higher level of free radical production in vivo, which in turn causes oxidative stress. Triglyceride and cholesterol levels significantly increased in our research rats fed an HFSD diet, indicating hypertriglyceridemia and hypocholesterolemia. When given to hyperlipidemic rats, Daidzein dramatically reduced lipid components such TGs, TC, VLDL, and LDL while raising HDL levels. Numerous pieces of data suggest that animals fed an HFSD diet have decreased antioxidant enzyme activity. Rats fed HSD in this study exhibit severe oxidative stress, as seen by lower serum SOD and GSH levels. In rats given an HFSD diet, oral Daidzein treatment restored these enzymes' activity. Histopathology of the liver of rats given HFSD revealed characteristics such as macrovesicular steatosis with distinct fat vacuoles and microvesicular steatosis with varyingly enlarged hepatocytes with extremely fine fat vacuoles. The ability of daidzein to preserve the histoarchitecture of liver cells was demonstrated by the significant changes observed in the rats fed HFSD when compared to the group that received only HFSD treatment.

CONCLUSION

In conclusion, the study demonstrates the protective effects of daidzein against obesity and cardiovascular complications induced by a high fat/high sucrose diet in rats. Daidzein treatment significantly reduced oxidative stress markers, improved lipid profiles, enhanced glucose tolerance, and showed hepatoprotective effects. While it did not affect body weight gain or food intake, daidzein's antioxidant properties were evident in the restoration of GSH and SOD levels. Histopathological examination confirmed its beneficial effects on liver tissue. These findings suggest daidzein's potential as a therapeutic agent for managing obesity-related metabolic disorders and cardiovascular complications, warranting further investigation into its mechanisms of action and long-term efficacy and safety.

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