대한예방한의학회지 제15권 제1호(2011년 4월)
Kor. J. Oriental Preventive Medical Society 2011 ; 15(1) : 29-36

Hypoglycemic and hypolipidemic effects of *Allium victorialis* leaf extract in high fat diet supplied mice

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Abstract

The hypoglycemic and hypolipidemic effects of *Allium victorialis* (AV) leaf methanol extract were evaluated in a high fat diet (HFD) supplied mice. Changes on the serum glucose, total cholesterol, triglyceride, low density lipoprotein and high density lipoprotein were examined. The effects were compared with those of a group given 250 mg/kg of metformin. After 91 days of a continuous HFD supply, the mice were showed marked hyperglycemia and hyperlipemia. However, these hyperglycemia and hyperlipemia induced by the HFD were inhibited by the AV extract treatment at the three different doses (62.5, 125 and 250 mg/kg). The results suggest that the AV methanol extract is beneficial for improving the diet-induced hyperlipidemia and hyperglycemia in humans.

Key words : *Allium victorialis*, hypoglycemic effects, hypolipidemic effects, high fat diet, mouse

· 접수 : 2011년 2월 9일 · 수정접수 : 2011년 4월 13일 · 채택 : 2011년 4월 26일
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1. Introduction

Recently, there has been a worldwide increase in the incidence of obesity associated with metabolic syndrome known as type 2 diabetes, the development of which appears to be the result of high-caloric diet intake and physical inactivity.[1] Obesity in mice can be developed by feedingthem a high fat diet (HFD), and the obese mice show the characteristics of hyperglycemia, insulin resistance, hepatic steatosis, mild diabetic nephropathy and hypolipidemia.[2-4] HFD-induced hyperglycemia in mice occurs as a result of the development of obesity after protracted access to a HFD. HFD-induced animal models show mild obesity with hyperglycemiaand hyperlipidemia, and has been used for developing preventive agents for metabolic syndromes.[5] Many oral antidiabetic medicines, including the thiazolidinediones and metformin, are currently used or have been developed to improve insulin resistance. Metformin inhibits hepatic glucose production by reducing the rate of gluconeogenesis,[6] and effectively inhibits high-fat induced hyperglycemia and hyperlipidemia in mice.[5] In the present study, metformin was used as a reference drug.

The pharmacological agents currently available for metabolic syndrome have a number of limitations, such as adverse effects and high rates of secondary failure.[7] Therefore, patients with metabolic syndrome and healthcare professionals are increasingly considering complementary and alternative approaches, including the use of medicinal herbs. *Allium victorialis* var. *platyphyllum* (Liliaceae ; AV) is an edible wild herb that has been used to treat heart failure and gastritis. Until now, AV extracts have been reported to have a range of pharmacological activities ; anti-arteriosclerosis activity,[8] anti-cancer activity,[9] antioxidant activity,[10] antidiabetic activity on streptozotocin-induced type I diabetic rats,[11] and nephroprotective and hepatoprotective effects on HFD supplied mice.[12] However, there are no reports of the effects of AV extracts on hyperlipidemia and hyperglycemia in mouse induced by HFD supply. Therefore, this study evaluated the hypolipidemic and hypoglycemic effects of an AV methanol extract in mice supplied with a HFD to test the hypothesis that an AV extract can improve diet-induced hyperlipidemia and hyperglycemia in humans.

2. Materials and Methods

2.1. Experimental animals

Female ICR mice (6-wk old upon receipt, SLC, Japan) were used in this study after allowing 7 days acclimatization. The animals were allocated four per polycarbonate cage in a temperature (20~25°C) and humidity (40~45%) controlled room. The mice were placed in a 12hr : 12hr light : dark cycle with a normal rodent pellet diet and water supplied *ad libitum* during acclimatization. Forty eight mice (8 per group) were selected for the experiments based on their body weight at 7 days after initiating the HFD supply to selected adapted mice. This study was carried out with prior approval of the animal Ethical Committee, The University of Daegu Haany Uni-

versity.

2.2. Experimental design

The experimental groups were divided into a normal pellet diet (Superfeed Co., Korea) supplied an intact control diet, HFD (Diet Research, USA ; Table 1) supplied control, 250 mg/kg of metformin-administered group, and 62.5, 125 and 250 mg/kg AV extract groups. Forty mice were supplied with a HFD. The remaining eight rats were used as the intact control. The dosing of the test articles was initiated from 7 days after HFD supply, and each sample was administered orally once a day over a 12 weeks period.

2.3. Preparation of AV extracts

The AV was purchased from Cho-Heung Pharmaceutical Ind. Co. (Korea) after confirming its morphology under a microscope. The methanol AV extracts(yield 11.00 %) were prepared from AV by routine methods using a rotary vacuum evaporator (Lab. Camp, Korea) and a programmable freeze dryer (Ilshin Lab., Korea). The dark black mucous AV extract was stored in a desiccator to protect them from light and moisture. The AV extract was dissolved in injectable distilled water and administered by an oral gavage at a dose of 5 ml/kg.

Table 1. Formulas of normal and high fat diet used in this study

	Normal pellet diets (g/kg diet) a	High fat diets (g/kg diet) b
Ingredient		
Casein	200	200
L-Cystein	3	3
Corn starch	150	72.8
Sucrose	500	172.8
Cellulose	50	50
Soybean Oil	50	25
Lard	**0**	**177.5**
Mineral mixture	35	10
Vitamin mixture	10	10
Choline bitartrate	2	2
Energy (kcal/g)	**0.21**	**4.73**
Protein (% kcal/kg)	13.3	20
Carbohydrate (% kcal/kg)	47.4	35
Fat (% kcal/kg)	**8.0**	**45**
Fiber (% kcal/kg)	8.0	8.0

[a] normal rodents pellet diet (Superfeed Co., Korea) were used as normal diets
[b] 45%Kcal/Fat pellet diets (D12451 ; Diet research, USA)

2.4. Detection of blood glucose level

At 84 days after treatment, blood was collected from the vena cava. The collected blood samples were deposited into a NaF glucose vacuum tube (Becton Dickinson, USA) and the plasma was separated. The blood glucose levels were determined using an automated blood analyzer (Hemagen Analyst ; Hemagen Diagnostic, USA).

2.5. Serum biochemistry

At sacrifice, approximately1ml of venous blood was collected from the vena cava under anesthesia. All blood samples were centrifuged at 15,000 rpm for 10min at room temperature using a clotting activated serum tube. Subsequently, the total serum cholesterol, triglyceride and low density lipoprotein (LDL) levels were detected using an automated blood analyzer (Hemagen Analyst ; Hemagen Diagnostic, USA), and the high density lipoprotein (HDL) levels were measured using another type of automated blood analyzer (AU400 ; Olympus, Japan).

2.6. Statistical analysis

The results are expressed as the mean ± standard deviation. Statistical analyses were carried out using SPSS for Windows (Release 14.0K, SPSS Inc., USA). Multiple comparison tests for different dose groups were conducted. The variance homogeneity was examined using the Levene test. If the Levene test indicated no significant deviations from variance homogeneity, the data was analyzed by one way ANOVA test followed by a least-significant differences multi-comparison test to determine the pairs of group comparisons that were significantly different. A non-parametric comparison test, Kruskal-Wallis H test was conducted in the case of significant deviation from variance homogeneity in the Levene test. When a significant difference was observed in the Kruskal-Wallis H test, a Mann-Whitney U test was carried out to determine the specific pairs of group comparisons that were significantly different. A p value < 0.05was considered significant.

3. Results

3.1. Changes in the blood glucose levels

The blood glucose levels were significantly higher in the HFD control than theintact control ($p<0.01$). However, the blood glucose levels were significantly ($p<0.01$ or $p<0.05$) lower in the metformin and AV extract-treated groups (at all three doses) than the HFD control (Fig 1).

3.2. Changes in the serum biochemistry

The serum total cholesterol, triglyceride and LDL levels were significantly higher in the HFD control than theintact control, but the serum HDL levels were significantly lower ($p<0.01$). On the other hand, the serum total cholesterol, triglyceride and LDL were significantly ($p<0.01$ or $p<0.05$) lower in all the administered groups with slightly increases in the serum HDL levels compared to the HFD control (Table 2).

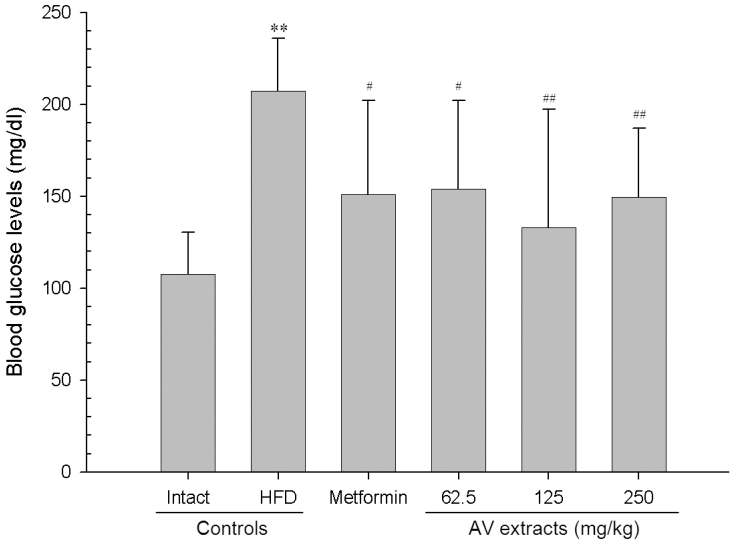

Fig. 1. Effect of the AV extracts on the blood glucose levels on the HFD supplied mice. Note that blood glucose levels were significantly decreased in the metformin and AV extract-treated groups as compared with HFD control The values are expressed as the Mean S.D. of eight mice. $^{*}p<0.05$ vs intact control ; $^{**}p<0.01$ vs intact control ; $^{\#}p<0.05$ vs HFD control ; $^{\#\#}p<0.01$ vs HFD control.

4. Discussion

Many animal models have been used to develop new drugs for metabolic syndrome but most showed serious obesity and hyperglycemia, making them suitable for evaluating treatments for established diabetes.[13] On the other hand, HFD-induced animal models show mild obesity and hyperglycemia, and are good for developing preventive agents for metabolic syndromes.[5] In the present study, HFD supplied mice showed marked hyperglycemia and hyperlipemia - increases in the total serum cholesterol, triglyceride and LDL levels with decreases in the HDL levels. This means that hyperglycemia and hyperlipidemia were induced by 91 days of a continuous HFD supply. However, thesehyperglycemia and hyperlipemia induced by the HFD supply were inhibited by an 84 day continuous treatment with the AV extracts at three different doses. Moreover, 125 mg/kg of the AV extracts showed similar effects to those of metformin 250 mg/kg.

Hyperglycemia is the main sign of diabetes and should be controlled.[14] HFD supplied mice have been used as an animal model for type 2 diabetes.[2, 4] In the present study, the AV extracts clearly inhibited the increases in the serum glucose level induced by the HFD. The hypoglycemic effects of 125 mg/kg of the AV extracts were similar to those of metformin 250 mg/kg.

Generally, the most critical problem in hyperlipemia is the increase in serum LDL, triglyceride and total cholesterol levels with a

Table 2. Effect of the AV extracts on the serum lipid levels in HFD supplied mice

Groups	Serum levels (mg/dl)			
	Total cholesterol	Triglyceride	LDL	HDL
Controls				
Intact	62.8811.19	49.0014.10	11.384.57	71.5511.46
HFD	145.6333.12**	112.3838.31**	18.382.83**	49.7513.82*
Reference				
Metformin	86.1319.74##	69.6318.49#	9.571.98##	61.3813.03
AV extracts				
62.5mg/kg	89.3824.68##	53.3812.35##	13.756.27#	61.0318.60
125mg/kg	98.3821.17##	43.6320.30##	10.382.20##	63.5520.63
250mg/kg	90.3835.03##	50.1310.19##	11.003.30##	63.0523.33

Values are expressed as Mean ± S.D. of eight mice * $p < 0.05$ *vs* intact control ; ** $p < 0.01$ *vs* intact control ; # $p < 0.05$ *vs* HFD control ; ## $p < 0.01$ *vs* HFD control

decrease in the HDL levels.[15-17] The efficacy of hypolipemic agents is generally evaluated based on the decrease in the serum LDL triglyceride and total cholesterol with the increase in HDL levels.[18] In the present study, dramatic increases in the serum LDL, triglyceride and total cholesterol levels were detected with a concomitant decrease in the serum HDL levels after a 91 day HFD. However, these were inhibited markedly by the extracts treatment, which is considered direct evidence of its hypolipemic effects.

There is considerable evidence on the role of free radicals in the etiology of diabetes and the altered antioxidant defenses in diabetes.[19] Oxidative stress in diabetes coexists with a decrease in the antioxidant status,[20] which can exacerbate the deleterious effects of free radicals. The generation of reactive oxygen species (oxidative stress) plays an important role in the etiology of diabetic complications,[21] and increased systemic oxidative stress has been found in HFD induced obese mice.[22] Therefore, the AV extract has hypoglycemic and hypolipidemic effects on HFD supplied mice resulting from its antioxidative and free radical scavenging activity, which have already been reported.[10] However, further studies will be needed to determine the precise mechanism of the favorable effects of AV extracts.

In conclusion, a methanol extract of AV showed relatively good inhibitory effects on the HFD-induced hyperlipidemia and hyperglycemia. The results suggest that the administration of the AV extracts can improve the diet-induced hyperlipidemia and hyperglycemia in humans.

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