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Potential on Hypotriglyceridemic Effect of Chloroform-Methanol Extract of Adlay Diabetic Rats

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ABSTRACT

The purpose of this study was to investigate the potential hypolipidemic effect of adlay extract in streptozotocin-induced diabetic rats. Fifty six rats were fed either a control diet or adlay extract diets of : Methanol 1%(M1%) ; Methanol 2%(M2%) ; Methanol 4%(M4%) ; Chloroform-Methanol 1%(CM1%) ; Chloroform-Methanol 2%(CM2%) ; Chloroform-Methanol 4%(CM4%) for 3 weeks. The amount of extracts added was 1%, 2% or 4% by diet weight respectively. The levels of glucose, total cholesterol(TC), high-density-lipoprotein cholesterol (HDL-C), free-fatty acid(FFA) and triglyceride(TG) in plasma, liver and skeletal muscle were compared. Among diabetic rats, there were no significant differences in the plasma level of glucose and TC regardless of a different extraction procedure or different amount of extracts added. While the plasma TG level tended to increase with times passed in diabetic control group, was not increased with times passed in CM groups and was significantly lower in CM groups at 3rd week. Compared to the diabetic control group, the levels of FFA tended to be lower in all M groups and were not different in all CM groups. The levels of HDL-cholesterol were not different in all M groups and were significantly lower in all CM groups than diabetic control group. Compared to diabetic control group, liver triglyceride level was lower in M4% group and no significant difference was seen in M1%, M2% and all CM groups. Muscle triglyceride level tended to be lower in M1%, M2% and CM2% group and significantly was lower in M4% and CM4% group. Thus, it can be suggested that a CM extract of adllay could have a potential hypotriglyceridemic effect on diabetic subjects. (*Korean J. Nutrition* 31(5) : 921~926, 1998)

KEY WORDS : adlay extract · hypotriglyceridemic effect · diabetes.

Introduction

Adlay(Coix Lachryma-Jobi) has been utilized as a diuretic, stomachic, analgesic, antispasmodic and hypoglycemic agent in oriental folk medicine without scientific verification. Recently, physiologically active substances have been isolated, possessing antiphlogistic, anti-tumor promoting activity[1-3]. However, no active constituent related to the treatment for diabetes other than dietary fiber from adlay has been isolated. Previously, we have reported that the hypoglycemic effect of adlay diet is not significant[4], but the steamed adlay and roasted adlay could have the potential on improving lipid profile of diabetes[5] when the amount of total fiber consumption is controlled. Also, oats which has the similar nutritional composition have been used successfully to lower blood lipids in several studies[6-9] and have been promoted as lipid-lowering food. Because the digestibility of starchy food is affected by the cooking methods[10,11] and

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it was also reported that an oil obtained from adlay seed, like lauric acid, myristic acid, oleic acid and palmitic acid elicit actual therapeutic effects in diabetes [12], we have investigated the potential curative activities of adlay on diabetes using oil extract employed different extractant as a part of our continuing studies on biological activities of natural products.

Materials and Methods

1. Animals and diets

Fifty six male Sprague-Dawley rats of 160-180g were divided into 8 groups ; control group, diabetic-control group, diabetic-Methanol 1%(M1%) group, diabetic-Methanol 2%(M2%) group, diabetic-Methanol 4%(M4%) group, diabetic-Chloroform-Methanol 1% (CM1%) group, diabetic-Chloroform-Methanol 2% (CM2%) group, diabetic-Chloroform-Methanol 4% (CM4%) group. Diabetes were induced by streptozotocin injection into the tail vein(45mg/kg body weight), which specifically affects the β-cell of pancreas[13] prior to initiating the experimental diets. Diabetic rats were confirmed with blood glucose level 24 hour after streptozotocin injection. Animals received adlay extract diet or control diet for 3 weeks. The control diet was a vitamin-free-casein-based semisynthetic diet which met AIN-76 recommendation[14][15]. The amount of extracts added was 1%, 2% or 4% by diet weight respectively. The calorie of adlay extract diet was manipulated to get similar calorie to that of control diet by substracting the amount of fat. The oily component of adlay was extracted with methanol or chloroform-methanol mixture. The methanol extraction procedure was as followes. The roasted adlay powder was provided by Yeonchon Agricultural Cooperative Association(Yeonchon, Korea). Two kilograms of the powder were extracted four times with 4 liter of methanol each time at boiling temperature. The filtered methanol extract was evaporated in vacuo and frozen dried. This procedure was repeated until sufficient crude extract was obtained to feed experimental groups. The chloroform-methanol extraction procedure was same as that of methanol extraction except that a mixing ratio of chloroform-methanol was 2 to 1. Prior to initiating the experimental diets, rats were given ad libitum access to the control diet for 1 week to adapt to the diet and the feeding schedule and the to bring all the rats to a similar metabolic status.

Table 1. The body weight(BW) change and feed efficiency ratio(FER)[1][2]of rats fed adlay extracts for 3 weeks

	BW change(g)	FER
Normal	96.2±17.4[a]	0.287±0.050[a]
Diabetes - Control	-5.7±25.4[b]	-0.013±0.056[b]
- M1%[3]	-11.3±13.2[b]	-0.030±0.035[b]
- M2%	-6.1± 9.9[b]	-0.051±0.024[b]
- M4%	-0.9±29.5[b]	-0.002±0.061[b]
- CM1%	-0.8±26.2[b]	-0.002±0.053[b]
- CM2%	3.5±15.2[b]	0.009±0.037[b]
- CM4%	2.8±25.3[b]	0.009±0.053[b]

1) Values are mean±SEM, n=6
2) Within a given column, those values with different superscripts are significantly different($p<0.05$)
3) M1%=1% methanol extract ; M2%=2% methanol extract ; M4%=4% methanol extract ; CM1%=1% chloroform-methanol extract ; CM2%=2% chloroform-methanol extract ; CM4%=4% chloroform-methanol extract

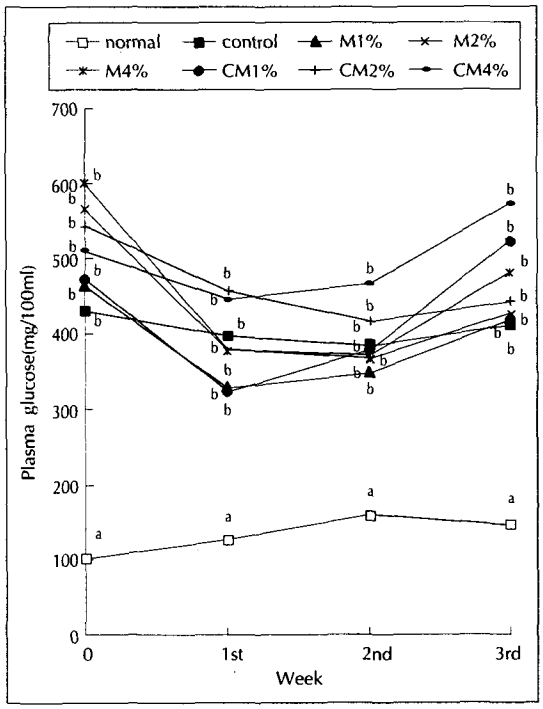

Fig. 1. The effect of Adlay. extract on the level of plasma glucose in fed rats. (Within a given column, those values with different superscripts significantly different at $p<0.05$; M1%=1% methanol extract ; M2%=2% methanol extract ; M4%=4% methanol extract ; CM1%=1% chloroform-methanol extract ; CM2%=2% chloroform-methanol extract ; CM 4%=4% chloroform-methanol extract)

2. Sample collection and analysis

All animals were weighed weekly and the food intake was measured daily. Blood was drawn from an eye vein in fed state weekly. Animals were anesthetized with ether and sacrificed by decapitation. Immediately following decapitation, blood was collected in heparinized tubes and centrifuged to separate the plasma. Organs were rapidly blotted-dry and weighed. Plasma and tissues were stored at −70℃ until analyzed.

Plasma glucose was analyzed with a commercial kit based on utilizing glucose oxidase(Youngdong Pharmaceutical Co., Korea). Plasma total-cholesterol was analyzed with a commercial kit based on utilizing cholesterol oxidase(Youngdong Pharmaceutical Co., Korea). After precipitation of LDL, VLDL and chylomicron with polyethyleneglycol HDL-cholesterol was analyzed with a commercial kit based on the same analytical method used to measure total cholesterol(International Reagent Co., Japan). Triglyceride was analyzed with a commercial kit based on Trinder method(Youngdong Pharmaceutical Co., Korea). Free fatty acid was analyzed with a commercial kit based on utilizing acyl CoA synthetase-acyl CoA oxidase(Eiken Chemical Co., Japan). Tissue samples were homogenized in cold sodium phosphate buffer(0.02M, pH 7.0). Aliquots of the tissue homogenates were analyzed with the same method as that of plasma. For statistical analysis, all data were first evaluated by analysis of variance. For those F values which were significant, the least significant difference test was performed. A P value <0.05 was considered to be statistically significant.

Results

At various points during the study, the diabetic status of the rats was evaluated, using plasma glucose

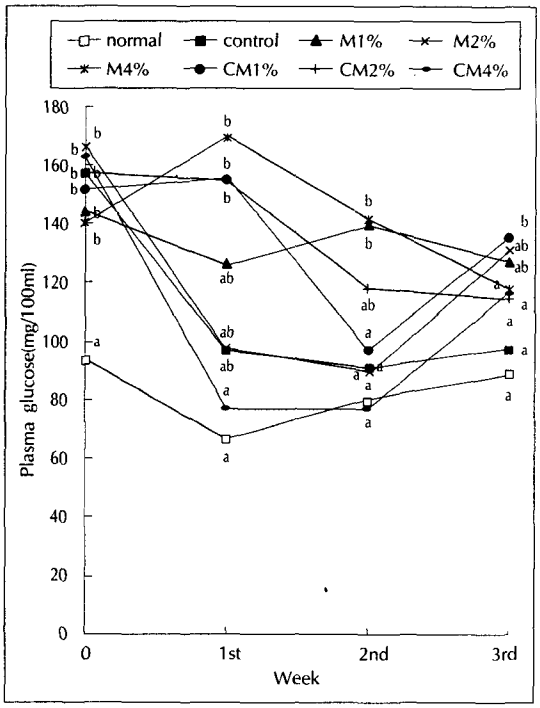

Fig. 2. The effect of Adlay. extract on the level of plasma total cholesterol in fed rats. (Within a given column, those values with different superscripts significantly different at $p<0.05$; M1%=1% methanol extract ; M2%=2% methanol extract ; M4%=4% methanol extract ; CM1%=1% chloroform-methanol extract ; CM2%=2% chloroform-methanol extract ; CM 4%=4% chloroform-methanol extract)

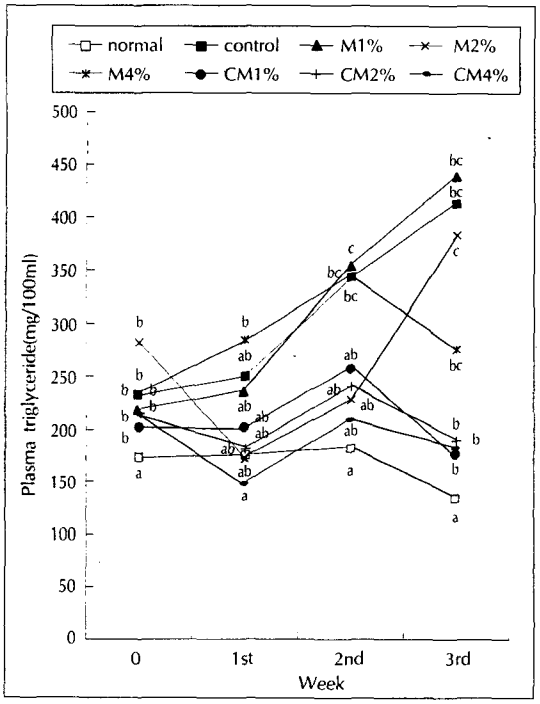

Fig. 3. The effect of Adlay. extract on the level of plasma triglyceride in fed rats. (Within a given column, those values with different superscripts significantly different at $p<0.05$; M1%=1% methanol extract ; M2%=2% methanol extract ; M4%=4% methanol extract ; CM1%=1% chloroform-methanol extract ; CM2%=2% chloroform-methanol extract ; CM 4%=4% chloroform-methanol extract)

Table. 2. The effect of adlay extract on the plasma lipid profile in rats fed adlay extracts for 3 weeks

	FFA[4] (mg/100ml)	TG (mg/100ml)	TC (mg/100ml)	HDL-C (mg/100ml)
Normal	429±88[a]	136±36[a]	90±10.3[a]	15.6±3.78[b]
Diabetes - Control	536±155[ab]	497±279[bc]	98±21.9[a]	12.2±4.15[b]
- M1%[3]	746±222[bc]	439±276[bc]	126±53.2[a]	16.6±5.32[b]
- M2%	997±139[b]	383±10[c]	128±47.4[a]	13.0±4.24[b]
- M4%	846±398[bc]	275±161[ab]	118±10.8[a]	9.2±6.97[b]
- CM1%	578±175[ab]	180±122[ab]	132±54.7[a]	0.4±0.55[a]
- CM2%	538±127[ab]	190±61[a]	115±12.0[a]	0.4±0.53[a]
- CM4%	580±250[ab]	184±77[a]	117±13.7[a]	0.3±0.46[a]

1) Values are mean±SEM, n=6
2) Within a given column, those values with different superscripts are significantly different($p<0.05$)
3) M1%=1% methanol extract ; M2%=2% methanol extract M4%=4% methanol extract ; CM1%=1% chloroform-methanol extract ; CM2%=2% chloroform-methanol extract ; CM4%=4% chloroform-methanol extract
4) FFA=free fatty acid ; TG=Triglyceride ; TC=Total cholesterol ; HDL-C=HDL-cholesterol

Table 3. The effect of adlay extract on the level of tissue triglyceride in rats fed adlay extracts for 3 weeks

	Liver	Muscle
Normal	18.5±4.1[a]	11.0±2.4[a]
Diabetes - Control	25.7±5.3[b]	26.5±11.6[b]
- M1%[3]	32.0±13.9[b]	17.8±4.7[ab]
- M2%	27.2±3.0[b]	18.2±8.2[ab]
- M4%	19.3±3.5[a]	14.1±6.8[a]
- CM1%	35.8±11.4[b]	23.4±4.2[b]
- CM2%	30.7±14.9[b]	16.3±5.7[ab]
- CM4%	25.8±16.9[b]	10.3±6.8[a]

1) Values are mean±SEM, n=6
2) Within a given column, those values with different superscripts are significantly different($p<0.05$)
3) M1%=1% methanol extract ; M2%=2% methanol extract M4%=4% methanol extract ; CM1%=1% chloroform-methanol extract ; CM2%=2% chloroform-methanol extract ; CM4%=4% chloroform-methanol extract

level, total body weight gain and feed-efficiency ratio. At week 3, the total body weight gain and FER of diabetic rats were significantly lower than those of normal rats(Table 1). Among diabetic rats, there were no differences between diabetic controls and diabetic-adlay extract group regardless of different extraction procedure or different amount of adding-extracts in body weight and FER. The diabetic group showed higher plasma glucose level than the normal group throughout the study. Among diabetic rats, the difference of the plasma glucose level was not significant due to a large standard deviation(Fig. 1).

Fig. 2 shows the effect of adlay extract on the change of the plasma total cholesterol level in fed rats. The plasma total cholesterol levels of all diabetic group were significantly higher than that of the normal group but these levels tended to decrease at the 1st week and remained until the 3rd week. The adlay extract group except CM4% even showed a higher plasma total cholesterol level than the diabetic control group. Fig. 3 shows the effect of adlay extract on the change of plasma triglyceride level in fed rats. The plasma triglyceride level of all methanol extract group tended to increase with time and showed no difference in the diabetic control group throughout the study. The plasma triglyceride level of the chloroform-methanol extract group did not increase with time. CM4% group showed a significant lower level at the 1st week. CM2% group showed a significant lower level at 3rd week and the CM1% group showed a similar tendency although the difference was not significant due to large standard deviation. The effects of adlay extract on plasma lipid profile in rats fed adlay extracts for 3 weeks were shown in Table 2. The levels of free fatty acid tended to be higher in all M groups and were not different in all CM groups than diabetic control group. The levels of triglyceride did not differ in all M groups and was lower in all CM groups than diabetic control group. The total cholesterol levels of all adlay extract groups tended to be even higher than the diabetic control group. The levels of HDL-cholesterol were not different in all M groups and were significantly lower in all CM groups than the diabetic control group. The effects of adlay extract on tissue triglyceride level in rats are shown in Table 3. Compared to the diabetic control group, the liver triglyceride level was lower in M4% group and no significant difference was seen

in the M1%, M2% and all of the CM groups. Muscle triglyceride levels tended to be lower in M1%, M2% and CM2% groups and were significantly lower in M 4% and CM4% group.

Discussion

The diabetic animals were considered to be severely diabetic throughout the study from the higher plasma glucose level with the lower growth rate and FER at various points during the study. Compared to diabetic control rats, the adlay extract group, regardless of different extractant or different amount of extracts added, showed no statistically significant difference on plasma glucose level. This result is consistent with a previous report[8] although is not consistent with that of Takahashi, et al.[12]. Thus, hypoglycemic effect of adlay which is reported by Takahashi, et. al. may be caused by a lipid-free residue rather than oily components in adlay.

Lipoprotein abnormalities play a major role in atherosclerosis. Plasma low density lipoprotein(LDL) abnormalities, decreased HDL and increased triglycerides contribute to accelerated atherosclerosis in diabetes. The finding that plasma total cholesterol was not improved in adlay extract group was consistent with the results of adlay oil diet[16], but not consistent with the previous results of adlay diet[9][17]. This discrepancy might be from the difference of soluble fiber contents between adlay diets and adlay extracts. Adlay contain water soluble-components as well as oily components and adlay extracts are mainly oily components. Total cholesterol have been reported to significantly decrease after the addition of soluble fiber to the diet compared to levels after a low fiber basal diet[18-22]. Water-soluble major polysaccharide from adlay was water soluble glucans[23]. Furthermore, HDL-cholesterol levels were not different in all M groups and were even lower in all CM groups. Also, plasma free fatty acid levels of all CM groups were not different from those of diabetic control groups and were even higher than those of diabetic control groups. Thus, it is not possible to suggest that adlay extract might have some hypocholesterolemic effects. However, the support for a potential on improving lipid profile is provided by the effect of adlay extract on triglyceride levels in plasma and muscle. Plasma trigyceride levels in CM groups were improved with the increase an amount of CM extract added throughout the study and muscle triglyceride levels of CM group were significantly lower than that of diabetic control group and close to that of normal group at 3rd week. This finding was consistent with the results of adlay diet[24][25]. Because the hypertriglyceridemia gives a higher risk of atherosclerotic cardiovascular disease on diabetic than nondiabetic indi-viduals[26] and the maintenance of desirable plasma lipid levels is the key to reduce the development of the complications in diabetes, it can be suggested that CM extract of adllay could have the potential of improving the lipid profile of diabetes in a clinical setting.

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