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総　　説

Study on Bioactive Components of *Anoectochilus formosanus* Hayata

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金線連の活性成分

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要　旨

本総説において以下の知見を得たのでこれらについて述べる。１)マウスを用いた金線連の薬理活性、特に肝障害改善作用について検討した。２)ボランテイアによる脂質関連障害の改善傾向を認めた。３)金線連の粗エキスから脂質代謝改善作用を指標として kinsenoside を単離し、本化合物が活性本体であることを明らかにした。

ラン科に属する金線連（*Anoectochilus formosanus* Hayata）は台湾から沖縄にかけて自生しており、高血圧、肺障害、肝障害、小児の発育障害等に用いられてきた。原料の枯渇から金線連の近縁種が代用として用いられるようになってきた。かかる現状から研究材料を確保する目的で、金線連の完熟種子を培地へ無菌的に播種し、発芽後幼植物を得た。このものを液体培地で培養し、植物体を得て実験材料とした。

金線連のエキスは四塩化炭素で誘導する肝細胞障害を抑制することが判明した。また、マウスの体重増加や肝重量増加を抑制した。さらにマウスの肝臓や血清中の脂質を低下させる作用が認められた。ボランテイアによる金線連エキスの臨床試験の結果、健常者には作用しないが、脂質やコレステロール、またその両者が高い患者に対しては VLDL や LDL、ALT、AST 値を下げることが明らかとなった。

培養金線連を抽出しカラムクロマトにて成分の精製単離を行い、８種の化合物を単離し構造を明らかにした。その中で量的にも多い kinsenoside についてはX−線解析を行い、絶対構造を明らかにした。

金線連エキスにつき脂質代謝を指標として分画を行い、kinsenoside がその活性本体であることを明らかにした。Kinsenoside を金線連エキス同様の評価系にて評価した結果、肝臓や子宮の脂質量低下作用があることが明らかとなった。脂肪肝を発症させたマウスに kinsenoside を与えて顕微鏡により調査した結果、0.2%の kinsenoside を与えることにより脂肪肝は改善されることが明らかとなった。

キーワード

Anoectochilus 属植物、ラン科、キンセノシド、抗高脂血漿、肝保護作用

Abstract

In this review we discuss the pharmacological activities of *Anoectochilus formosanus* in mice and the clinical evidences using volunteers, and the determination of an active component from *A. formosanus.*

The extract of *A. formosanus* showed significant activity in decreasing the levels of the cytosolic enzymes LDH, GOT and GPT, and the result demonstrated that *in vitro* cultured *A. formosanus* possessed prominent hepatoprotective activity against CCl_4-induced hepatotoxicity. In the test using aurothioglucose-induced obese mice, the extract of *A. formosanus* showed a significant antihyperliposis effect. The body and liver weights were significantly suppressed ameliorating TG levels in the liver and serum in the extract treated group. When the high TG volunteers were used, the administration of *A. formosanus* significantly decreased the levels of TC, VLDL and Apo E. The results of

the present study suggest that *A. formosanus* may be useful for improvement of lipid-metabolism and the prevention of atherosclerosis.

A. formosanus grown in wild and propagated by tissue culture contain ten compounds including a major known component, kinsenoside and two new components, 3-(*R*)-3-β-D-glucopyranosyloxy-4-hydroxybutanoic acid, and 2-(β-D-glucopyranosyloxymethyl)-5-hydroxymethylfuran, along with the known compounds, 1-*O*-isopropyl-β-D-glucopyranoside, (*R*)-(+)-3,4-dihydroxy-butanoic acid γ-lactone, 4-(β-D-glucopyranosyloxy) benzyl alcohol, (6*R*,9*S*)-9-hydroxy-megastigma-4,7-dien-3-one-9-*O*-β-D-glucopyranoside, and corchoionoside C. In an antihyperliposis assay using high-fat diet rats and in aurothioglucose-induced obese mouse, kinsenoside significantly reduced the weights of body and liver, and also decreased the triglyceride level in the liver compared to those of control rats.

Key words

Anoectochilus species, Orchidaceae, kinsenoside, antihyperliposis, hepatoprotective activity

Introduction

The *Anoectochilus* species (Orchidaceae) is perennial herbs which comprise more than 35 species that are widespread in the tropical regions, from India through the Himalayas and southeast Asia to Hawaii.[1] Several species have been used in Chinese folk medicines.[2] Among them, *A. formosanus* which is only found in Taiwan and Okinawa, has been used for hypertension, lung and liver diseases and underdeveloped children as a folk medicine.[3] It becomes evident that the natural resources of *A. formosanus* are becoming exhausted, therefore the other *Anoectochilus* species such as *A. koshunensis* and a different genus, *Goodyera* species are commercialized as substitutes used for the same purpose in the recent market.[4,5] From such situation we started to investigate the micropropagation of this species by tissue culture techniques, and worked on its chemical components and pharmacological profiles.[6–8]

Since we isolated a great amount of kinsenoside without its methyl ester by silica gel column chromatography eluting with chloroform-ethanol solvent system, it is easily suggested that some artificial conversion occurred during the separation procedure. This result inspired us to investigate the existence and concentrations of kinsenoside and its analogues in *Anoectochilus* species. Purified kinsenoside had antihyperliposis and hepatoprotective activity.

This review demonstrated the clinical evidences using volunteers will be also discussed.

1. Micropropagation of *A. formosanus* and its components

The ripened fruits of *A. formosanus* were sterilized, and seeds in fruits were cultured on 1/2 MS medium[9] supplemented with 1 mg/l 6-benzylaminopurine (BA) and 2 g/l peptone. After 30 days the seeds germinated and formed protocorms at 25℃. The seedlings, 6–8 cm in length, were transferred to 1/2 MS liquid medium to produce the multiple shoot complex. They were cultured in 1/2 MS liquid medium supplemented with 0.3 mg/l BA and 0.03 mg/l α-naphthaleneacetic acid (NAA) under shaking at 30 rpm. After 2 months they were transferred to 1/2 MS liquid medium without growth regulator and shaken for 4 more months. The regenerated plantlets were grown for 6–8 months as indicated in Fig.1. Since the wild and cultured *A. formosanus* had the completely same composition, the cultured plants were

Fig 1. Flowering of cultured *Anoectochilus formosanus* Hayata

used for further investigations.

The regenerated plants at three different stages of culturing : (a) multiple shooting, (b) shoot elongation and (c) rooting were analyzed. The results suggested that the cultured segments, a, b and c and the original plant had the same pattern of composition, although the concentrations were somewhat different. Therefore, it is evident that micropropagation of this species using plant biotechnological technique will be available to enable maintenance of this important crude drug.

2. Antihyperliposis activity of crude extract of *A. formosanus*

The hyperlipidemia and obesity following customs of livelihood is a major health problem in many countries with the escalation of obesity-related disease. The prevalence is increasing even in developing countries. This condition is associated with increased risk of cardiovascular, cerebrovascular, and type II diabetes. The magnitude of this health problem gives impetus to probe effective therapy, especially with natural crude drugs.

Liver, the key organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. It is well recognized that free radicals are critically involved in various pathological conditions such as cancer, cardiovascular disorder, arthritis, inflammation, and liver diseases. CCl_4 is biotransformed under the action of cytochrome P450 2E1 to the free radical trichloromethyl or peroxyltrichloromethyl.[10] These free radicals primarily affect centrolobular hepatocytes, binding to cell, nuclear proteins and DNA.[11] Therefore, the evaluation of the preventive action in liver damage induced by CCl_4 has been used widely as an indicator of the liver protective ability of drugs in general, even if CCl_4 liver injury resembles the damage due to acute viral hepatitis.[11] The extract of *A. formosanus* showed significant activity in decreasing the levels of the cytosolic enzymes LDH, GOT and GPT as indicated in Table 1, and the result demonstrated that *in vitro* cultured *A. formosanus* possessed prominent hepatoprotective activity against CCl_4-induced hepatotoxicity.

After a single injection of aurothioglucose, the mice showed a marked increase in body weight, and this method has been used widely as an indicator of the antihyperliposis of drugs in general. In the results of the test using aurothioglucose-induced obese mice,[12] the extract showed a significant antihyperliposis effect. There was no significant difference in food consumption between the normal and control or sample treated groups. This is in agreement with the literature.[12] Fig. 2 shows the body weights

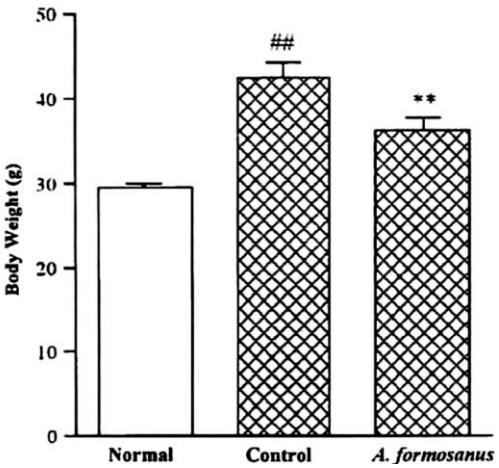
of normal mice, and of aurothioglucose-induced obese mice that were fed the HCD (as control) or the same diet containing 0.5% of the extract of *A. formosanus* after 8 weeks, respectively. A rapid weight increase was observed in the control group, while it was significantly suppressed in the extract treated group. The extract suppressed the liver weight increases (Fig. 3), significantly ameliorated TG levels in the liver and serum. Concentrations of TG in the liver and serum were significantly increased in the control group compared to the normal group, while these were significantly reduced in the extract of *A. formosanus* treated group (Fig. 4 and 5).

Concentrations of T-CHO in liver and serum in the control group were slightly higher than that in the normal group, but in the extract treated group, they were lower than in the control group although were not significantly different from the normal, control and extract treated groups (data not shown). No significant differences were noted between the normal, control and extract treated groups in serum levels of GOT

Table 1. Hepatoprotective activity of *Anoectochilus formosanus* crude extract on CCl_4-induced cytotoxicity in primary cultured rat hepatocytes

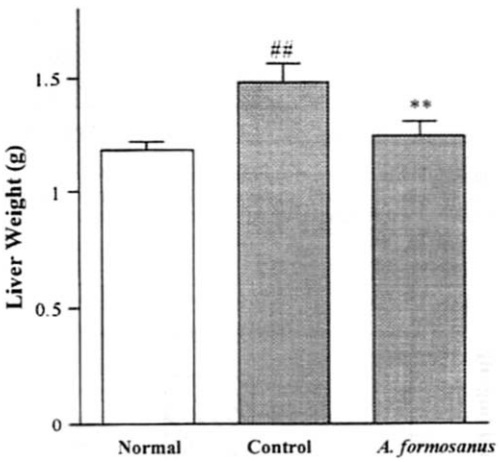
Group	Dose (μg/ml)	LDH (units/ml)	GOT (units/ml)	GPT (units/ml)
Normal		189.78 ± 6.18	55.45 ± 1.85	9.93 ± 3.04
Control	CCl_4 (5mM)	1,918.65 ± 43.98	480.73 ± 18.79	92.50 ± 2.48
Extract	1	1,678.83 ± 51.37*	314.71 ± 17.92*	77.20 ± 1.82*
	10	1,543.63 ± 90.13*	250.53 ± 14.00*	72.18 ± 2.93*
	100	1,294.75 ± 99.58*	200.25 ± 17.58*	59.90 ± 5.16*

Values are presented as means±SEM, $n = 4$
Significantly different from the control group, $^*p < 0.01$



##p < 0.01 significantly different from the normal group; **p < 0.01 significantly different from the control group

Fig 2. Effect of *Anoectochilus formosanus* crude extract on body weight of mice



##p < 0.01 significantly different from the normal group; **p < 0.01 significantly different from the control group

Fig 3. Effect of *Anoectochilus formosanus* crude extract on liver weight of mice

and GPT, but in the extract of *A. formosanus* treated group, the levels were lower than that in the control group (data not shown).

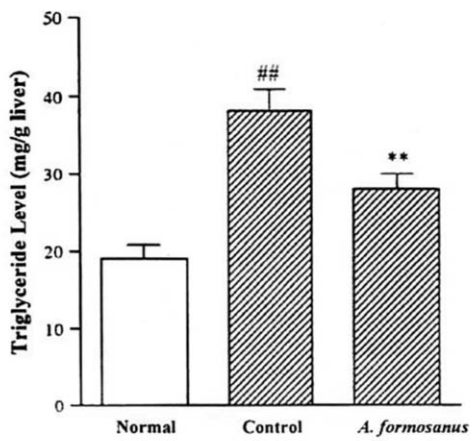
It has been reported that the change of the epididymal fat-pads corresponded to the total fat tissue in the body. From these results, it was believed that the body weight increase was suppressed by the improvement of lipid-metabolism, and it is suggested that the whole plants of in vitro cultured *A. formosanus* may be useful for the treatment for hyperliposis, especially for fatty liver. Moreover, the improvement of lipid-metabolism might be closely related to the hepatoprotective activity of *A. formosanus*.

3. Clinical evidences

The higher levels of TC and TG in serum than 220 mg/dl and 150 mg/dl, respectively, were considered to be hyperlipidemia. Since the VLDL and LDL levels are depend on the increases of TC and TG, it is considered that the high-TC or TG levels may possibly induce high-lipoprotein. Cholesterol status became to be considered as an important factor since the correlation has been established between the serum cholesterol level and risk of coronary heart disease as well as the severity of atherosclerosis.[13-16] Moreover, the increases of VLDL and LDL levels were also observed in the disease of obesity, diabetes and nephrosis.

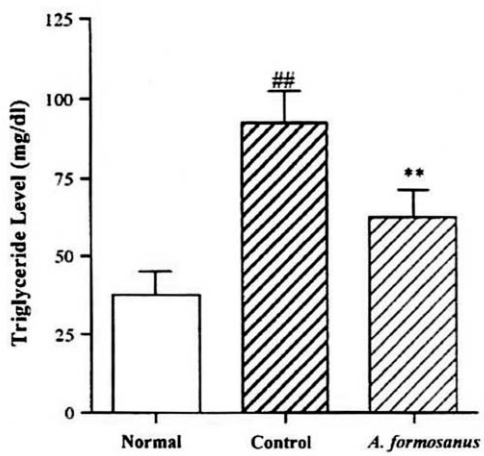
There were no significant differences in all anthropometry and serum biochemical parameters during 6 months of the administration period in normal health subjects. On the other hand, the level of Apo E decreased significantly after 12-months-treatment and that of VLDL did remarkably (Table 2).

When the high TG volunteers were used, the administration of *A. formosanus* significantly decreased the levels of TC, VLDL and Apo E (Table 3). The results of the present



##p < 0.01 significantly different from the normal group; **p < 0.01 significantly different from the control group

Fig 4. Effect of *Anoectochilus formosanus* crude extract on triglyceride level on the liver of mice.



##p < 0.01 significantly different from the normal group; **p < 0.01 significantly different from the control group

Fig 5. Effect of *Anoectochilus formosanus* crude extract on serum triglyceride level in mice

study suggest that *A. formosanus* may be useful for improvement of lipid-metabolism and the prevention of atherosclerosis. On the other hand, Nomura et al. confirmed the close correlation between hyperlipidemia, fatty liver and obesity by the previous investigation of nosographic study.[17)]

The levels of AST and ALT were significantly lower after 6-months-treatment, these levels, however, return to the original values. In the present study, 11 volunteers were high-ALT • AST levels, and they also have higher levels of BMI, VLDL and LDL compared to those of the normal volunteers. The levels of VLDL and Apo E were significantly lower after 12-months-treatment, al-

Table 2. Effect of *Anoectochilus formosanus* crude extract on the lipid metabolism in normal health subjects

	36 volunteers		21 volunteers	
	0 months	6 months	6 months	12 months
BMI (kg/m^2)	23.9 ± 3.2	23.9 ± 3.1	23.7 ± 3.1	23.8 ± 3.3
Percentage body fat (%)	28.1 ± 4.6	29.4 ± 4.1	28.5 ± 4.4	28.2 ± 3.9
TG (mg/dl)	90.1 ± 30.7	95.7 ± 41.4	93.9 ± 27.0	80.7 ± 25.9
TC (mg/dl)	177.9 ± 31.4	183.7 ± 33.0	189.3 ± 25.3	178.3 ± 28.8
HDL－C (mg/dl)	58.8 ± 13.2	54.5 ± 10.1	60.2 ± 15.1	54.7 ± 11.9
LDL (mg/dl)	100.1 ± 26.2	109.3 ± 26.9	106.3 ± 21.9	107.4 ± 26.9
VLDL (mg/dl)	124.9 ± 56.4	129.6 ± 61.8	129.9 ± 53.5	85.8 ± 36.5**
Apo A－I (mg/dl)	147.6 ± 20.7	149.3 ± 21.8	148.8 ± 22.2	147.1 ± 27.6
Apo B (mg/dl)	58.8 ± 13.2	79.9 ± 21.5	85.2 ± 17.0	93.5 ± 36.3
Apo E (mg/dl)	5.1 ± 1.6	5.1 ± 1.3	5.1 ± 1.4	4.2 ± 0.9*
AST (IU/l)	16.3 ± 5.5	16.9 ± 6.2	17.7 ± 6.5	18.2 ± 6.3
ALT (IU/l)	17.7 ± 10.5	18.5 ± 11.7	19.1 ± 12.2	18.9 ± 10.3
ALP (IU/l)	162.8 ± 53.2	160.4 ± 43.0	165.2 ± 63.9	159.0 ± 58.8
γ －GPT (IU/l)	30.8 ± 34.2	31.8 ± 37.6	27.5 ± 19.5	31.5 ± 34.0

Values ate presented as mean±SD
*$p < 0.05$, **$p < 0.01$: significantly different compared to values before treatment

Table 3. Effect of *Anoectochilus formosanus* crude extract on the lipid metabolism in high triglyceride subjects

	14 volunteers		7 volunteers	
	0 months	6 months	6 months	12 months
BMI (kg/m^3)	25.6 ± 4.2	25.5 ± 4.4	25.4 ± 1.8	25.3 ± 2.3
Percentage body fat (%)	26.8 ± 7.2	27.8 ± 8.1	26.7 ± 6.6	26.7 ± 9.2
TG (mg/dl)	242.4 ± 77.6	204.3 ± 48.2	207.6 ± 38.1	159.3 ± 34.7
TC (mg/dl)	208.2 ± 50.4	203.9 ± 36.4	223.7 ± 56.2	202.4 ± 47.6*
HDL－C (mg/dl)	45.9 ± 10.5	44.0 ± 11.5	49.9 ± 9.3	47.7 ± 10.2
LDL (mg/dl)	115.6 ± 46.0	119.0 ± 33.2	132.2 ± 48.8	122.9 ± 45.3
VLDL (mg/dl)	323.2 ± 87.3	321.7 ± 82.7	329.0 ± 77.7	165.0 ± 37.4**
Apo A－I (mg/dl)	141.6 ± 29.0	140.0 ± 23.7	146.1 ± 21.2	158.6 ± 33.6
Apo B (mg/dl)	104.6 ± 24.6	102.4 ± 24.4	108.1 ± 29.9	99.9 ± 53.9
Apo E (mg/dl)	7.2 ± 2.3	6.9 ± 1.4	7.3 ± 2.0	5.8 ± 1.2*
AST (IU/l)	24.6 ± 10.6	21.6 ± 6.7	23.4 ± 12.9	19.4 ± 6.5
ALT (IU/l)	31.7 ± 19.5	27.6 ± 18.4	27.9 ± 20.6	22.0 ± 10.5
ALP (IU/l)	204.2 ± 50.3	198.3 ± 54.6	198.0 ± 59.8	203.1 ± 77.9
γ －GPT (IU/l)	62.1 ± 65.2	52.9 ± 43.1	56.1 ± 74.1	55.4 ± 61.2

Values are presented as mean±SD
*$p < 0.05$, **$p < 0.01$: significantly different compared to values before treatment

though no difference was observed after 6 months-treatment (Table 4). The level of TG was decreased gradually during 12 months.

After administration of *A. formosanus* for 6 months, the levels of AST and ALT were significantly reduced, and after 12 months of treatment, the levels of TC, VLDL and LDL were also significantly reduced when used high TG and TC volunteers (Table 5). From these results, it was believed that the improvement of lipid-metabolism effect of *A. formosanus* might be closely related to the improvement of liver function. Liver, the key

Table 4. Effect of *Anoectochilus formosanus* crude extract on the lipid metabolism in high cholesterol subjects

	11 volunteers		
	0 months	6 months	12 months
BMI (kg/m^2)	25.8 ± 2.7	25.6 ± 3.1	25.6 ± 3.0
Percentage body fat (%)	25.6 ± 5.3	26.6 ± 5.8	25.4 ± 5.0
TG (mg/dl)	227.0 ± 54.7	166.6 ± 46.6	143.7 ± 50.3
TC (mg/dl)	248.5 ± 24.2	239.9 ± 18.5	235.8 ± 25.7
HDL－C (mg/dl)	53.9 ± 12.5	50.3 ± 13.0	53.1 ± 14.8
LDL (mg/dl)	156.3 ± 36.5	155.2 ± 24.8	160.3 ± 33.8
VLDL (mg/dl)	289.9 ± 126.3	282.0 ± 132.6	150.0 ± 53.4*
Apo A－I (mg/dl)	153.2 ± 31.2	146.0 ± 27.1	156.6 ± 39.5
Apo B (mg/dl)	125.2 ± 14.5	120.0 ± 20.5	128.0 ± 56.3
Apo E (mg/dl)	7.2 ± 2.9	6.9 ± 1.5	5.9 ± 1.5*
AST (IU/l)	27.6 ± 10.0	21.3 ± 6.8*	24.9 ± 9.5
ALT (IU/l)	35.3 ± 19.6	27.3 ± 14.4*	35.6 ± 18.4
ALP (IU/l)	208.6 ± 53.8	207.0 ± 48.4	211.7 ± 56.5
γ－GPT (IU/l)	84.9 ± 71.0	71.5 ± 61.8	78.4 ± 89.3

Values are presented as mean±SD
*$p < 0.05$, **$p < 0.01$: significantly different compared to values before treatment

Table 5. Effect of *Anoectochilus formosanus* crude extract on the lipid metabolism in high triglyceride and cholesterol subjects

	5 volunteers		
	0 months	6 months	12 months
BMI (kg/m^2)	25.2 ± 2.2	24.7 ± 2.1	23.7 ± 1.5
Percentage body fat (%)	25.0 ± 5.0	25.4 ± 5.5	22.6 ± 5.9
TG (mg/dl)	209.0 ± 46.7	186.8 ± 43.9	160.8 ± 42.1
TC (mg/dl)	265.4 ± 23.5	238.2 ± 27.0*	237.5 ± 12.5*
HDL－C (mg/dl)	52.6 ± 10.2	48.8 ± 10.4	53.0 ± 10.5
LDL (mg/dl)	171.0 ± 22.7	152.0 ± 25.7*	143.5 ± 39.1*
VLDL (mg/dl)	323.8 ± 103.2	363.0 ± 118.4	170.3 ± 28.1**
Apo A－I (mg/dl)	154.2 ± 20.9	145.8 ± 24.9	171.3 ± 39.8
Apo B (mg/dl)	130.2 ± 13.4	118.6 ± 23.9	107.5 ± 74.6
Apo E (mg/dl)	7.6 ± 2.2	7.6 ± 1.5	6.4 ± 1.1
AST (IU/l)	30.6 ± 13.5	23.2 ± 8.0*	22.3 ± 7.3*
ALT (IU/l)	40.0 ± 27.5	29.2 ± 19.6*	23.0 ± 14.2*
ALP (IU/l)	210.8 ± 69.2	203.2 ± 48.9	199.0 ± 61.5
γ－GTP (IU/l)	96.2 ± 93.7	71.6 ± 57.3	70.8 ± 81.8
Glucose (mg/dl)	100.8 ± 13.4	101.4 ± 21.2	89.7 ± 7.5
HbA1C (%)	5.7 ± 0.8	5.7 ± 0.6	5.3 ± 0.3

Values are presented as mean±SD
*$p < 0.05$, **$p < 0.01$: significantly different compared to values before treatment

organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. The effect of *A. formosanus* on liver function will be further investigated.

In general *A. formosanus* has been used 4 to 40 g of fresh weight per day, although the present study only used a very lower dose for volunteers (450 mg/day). The acute toxicological effects of water extracts of *Anoectochilus* species including *A. formosanus* were administered in single dose of 5,000 mg/kg p.o.. No gross abnormalities were found in any organs of the treated animals at the necropsy, but the change of body weight of tested rats showed significant decrease in male and female rats at 7 and 14 days. It becomes evidet that *Anoectochilus* species were non-or less toxic products.

4. Chemical composition of cultured *A. koshunensis*

The dried whole plants cultured were extracted with MeOH at room temperature, and the concentrate was suspended in H_2O and partitioned successively with $CHCl_3$ and *n*–BuOH. The residues obtained from the H_2O layer and the *n*–BuOH layer were separately subjected to normal-phase and reversed-phase silica gel column chromatography to give ten compounds. The structures of the known compounds were identified as glucose, sucrose, kinsenoside (1),[18] 3-(*R*)-3-β-D-glucopyranosyloxy-4-hydroxybutanoic acid (2),[6,7] 1-*O*-isopropyl-β-D-glucopyranoside (4),[8] (*R*)-(+)-3,4-dihydroxybutanoic acid γ-lactone (5),[18] 4-(β-D-glucopyranosyloxy) benzyl alcohol (6),[19] (6*R*,9*S*)-9-hydroxy-megastigma-4,7-dien-3-one-9-*O*-β-D-glucopyranoside (7),[20] and corchoionoside C (8),[20] by comparing their spectral data with those previously reported.

The positive ion FAB mass spectrum of unknown component, 2 showed a $[M+H]^+$ peak at *m/z* 283 suggesting that it had the molecular formula $C_{10}H_{18}O_9$. The ^{13}C NMR spectrum indicated the existence of a free carboxylic acid group (δ 179.6), an oxygenated carbon at δ 66.0 and a hexose moiety at δ 104.1. The ^1H NMR spectrum showed some readily assignable signals, such as two methylene groups δ 2.38 (^1H, *dd, J*=14.8, 5.9 Hz), 2.46 (^1H, *dd, J*=14.8, 7.3 Hz) and δ 3.59 (^1H, *dd, J*=12.5, 5.9 Hz), 3.64 (^1H, *dd, J*=12.5, 4.3 Hz), and a methine signal 4.12 (*dddd, J*= 7.3, 5.9, 5.9, 4.3 Hz). These data suggested the presence of 3,4-dihydroxy butylic acid in 2. When 2 was exposed to mild acid conditions, compound 3 was obtained. From the above evidence, the structure of 2 was determined to be 3-*O*-β-D-glucopyranosyl-(3*R*)-4-dihydroxy butanoic acid. From these results the stereochemistry of the C–3 hydroxyl group of 2 was confirmed to be *R*. Fig. 6 indicated the structures of components isolated from cultured *A. koshunensis*.

In order to confirm the exact structure of kinsenoside, X-ray diffraction analysis of the corresponding peracetate was investigated. The structure of kinsenoside (1), including the stereochemistry on the hydroxyl group at C–3 was identified unambiguously to be 3-*O*-β-D-glucopyranosyl-(3*R*)-4-dihydroxy butanolide, isolated from *A. koshunensis* and named as kinsenoside by Ito et al.,[18] as indicated in Fig. 7.

5. Antihyperliposis and hepatoprotective active compound, kisenoside

The crude extracts of in vitro cultured *A. koshunensis* were purified guided by anti-hyperliposis assay using high-fat diet rats to isolate kinsenoside as an active component.

In an assay for anti-hyperliposis effect using high-fat diet rats, kinsenoside significantly ameliorated the TG level in liver. The liver and body weights were lower than those of control group. Table 6 shows the change in body weight and liver weight when 6-week-old male SD rats were fed HFD (to make hyperlipemia model rats) and the same diet plus oral administration of kinsenoside [(50 mg/kg (being equal to 300–400 mg dried whole plant of *A. formosanus*) and 100 mg/kg (being equal to 600–800 mg plant)] for 6 days, respectively. The body weight increase was seen in the normal group and control group, although no difference was observed between these two groups. However, weight was suppressed in kinsenoside administered groups. Especially, the 100 mg/kg kinsenoside administered group exhibited significantly lower weight than the control group.

The liver weight of the control group was significantly higher than that of the normal group. However, the liver weight of both the 50 mg/kg kinsenoside group and 100 mg/kg kinsenoside group was significantly lower than that of the control group.

Fig. 8 shows the value of TG in liver. TG, a neutral lipid, is a risk factor implicated in obesity and other diseases. The TG level in liver of the control group was signifi-

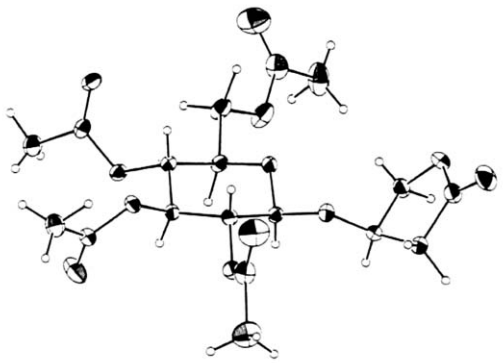


Fig 7. X-ray analysis of peracetyl-kinsenoside

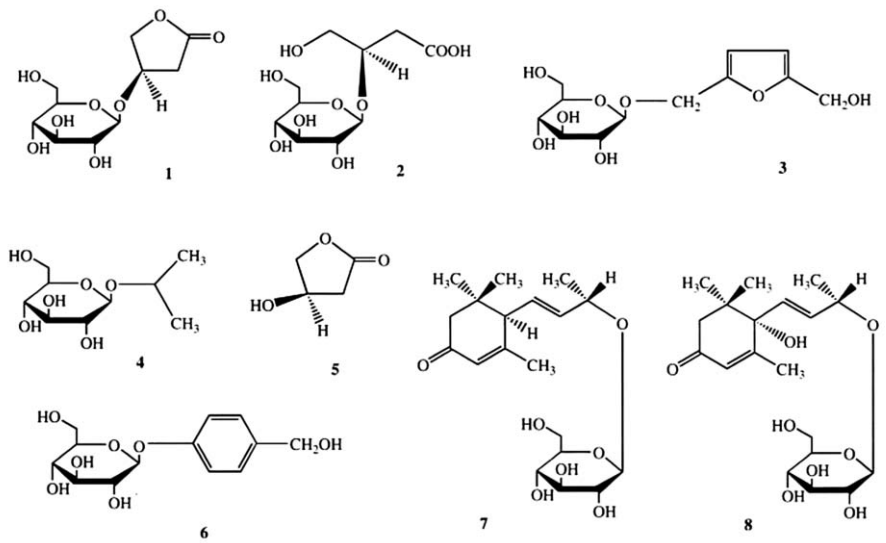


Fig 6. Structure of components isolated from *Anoectochilus formosanus*

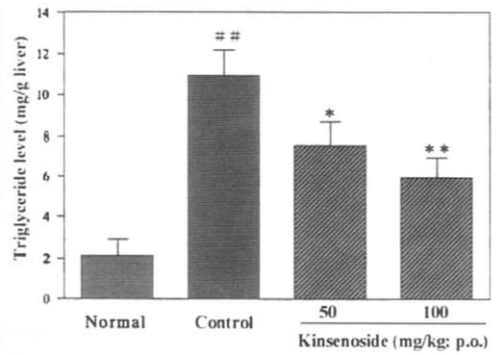
cantly higher than in the normal group. The levels in the 50 mg/kg kinsenoside group and the 100 mg/kg kinsenoside group were significantly lower than the control group. When compared to TG concentration per liver protein, the same result was obtained.

The effect of kinsenoside on anti-hyperliposis was also examined by using aurothioglucose-induced obese mouse.[20, 21] There was no significant difference in food consumption between normal and the control or kinsenoside treated groups. This is in agreement with the literature.[22, 23] Diet amounts during the period were 3.93 g/d for the normal mouse, 4.18 g/d for the control mouse, 4.23 g/d and 4.09 g/d for kinsenoside treated mouse. Fig. 9 shows the change in body weight for normal mice, and when aurothioglucose-induced obese mice that were fed the HFD (as control) or the same diet containing 0.1% or 0.2% kinsenoside for 6 weeks, respectively. Rapid weight increase was observed in the control group, while it was suppressed in the kinsenoside treated groups. Especially, the 0.2% kinsenoside administered group exhibited significantly lower weight than the control group. The weight increases of liver and uterine fat-pads were also observed in the control group, while in the kinsenoside treated groups, they were significantly de-

Table 6. Hepatoprotective activity of kinsenoside on the body and liver weight in rats

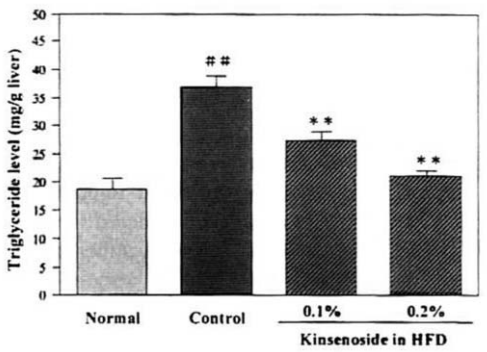
Group	Dose (p.o.) (mg/kg)	Body weight (g)	Liver weight (g)
Normal	—	212.32 ± 2.24	6.80 ± 0.18
Control	—	213.19 ± 4.23	8.81 ± 0.31##
Kinsenoside	50	204.10 ± 1.97	7.72 ± 0.18**
Kinsenoside	100	202.18 ± 2.39*	7.08 ± 0.28**

$p < 0.01$ versus normal group ; * $p < 0.05$, ** $p < 0.01$ versus control group



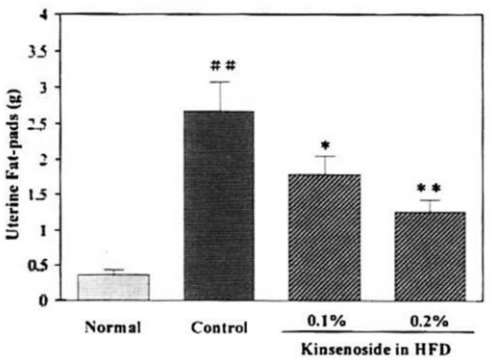
##p < 0.01 significantly different from the normal group; **p < 0.01 significantly different from the control group
*p < 0.1 significantly different from the control group

Fig 8. Effect of kinsenoside on triglyceride level in liver of rats



##p < 0.01 significantly different from the normal group; **p < 0.01 significantly different from the control group

Fig 9. Effect of kinsenoside on the triglyceride level in liver after aurothioglucose-induced obese mice after 6 weeks



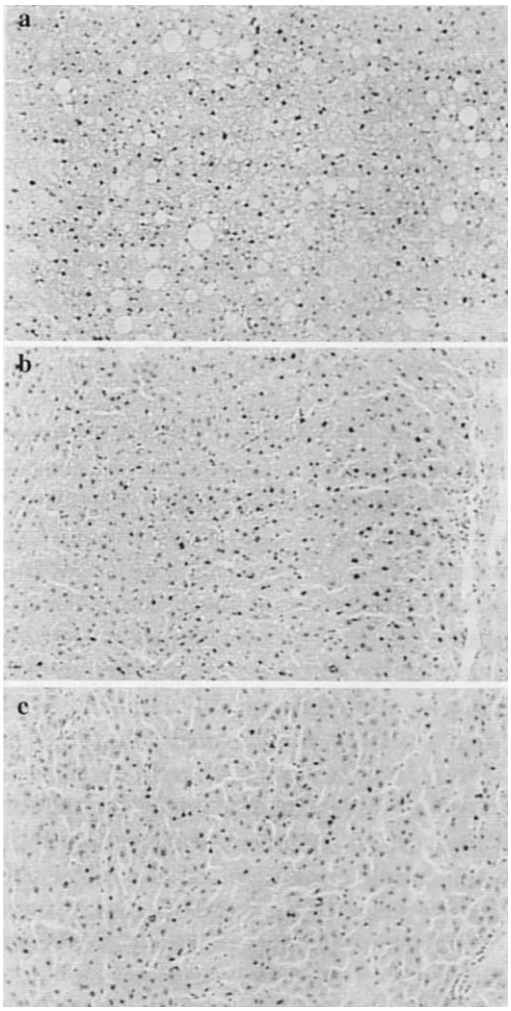
##p＜0.01 significantly different from the normal group; **p＜0.01 significantly different from the control group
*p＜0.1 significantly different from the control group

Fig 10. Effect of kinsenoside on uterine fat pads of aurothioglucose-induced obese mice after 6 weeks

creased (Fig. 10). The livers in the control group were considerably swollen, and the color and luster were those of fatty liver. These phenomena were not observed in the kinsenoside treated groups.

Fig. 11 shows the typical histological views of the liver in the mice being fed the HFD and the diet with kinsenoside(0.1% and 0.2%, respectively) after 6 weeks. A great deal of accumulated fatty drops was observed in the control group, while it was significantly decreased in the kinsenoside administered groups. It is considered that the body weight increase was suppressed by the improvement of lipid-metabolism.

The findings of the present study indicate kinsenoside may be useful for the treatment for hyperliposis. In aurothioglucose-induced obese mouse, kinsenoside suppressed the body and liver weight increase, significantly ameliorated the triglyceride level in the liver, and also reduced the deposition of uterine fat-pads.



a: control group b: 0.1% kinsenoside group c: 0.2% kinsenoside group

Fig 11. Photomicrographs of histophasological changes showing the effect of kinsenoside in the liver of aurothioglucose-induced obese mice after 6 weeks

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