Table 2 Final body weight, food intake and each tissue weight

	Young (seven weeks old)		Adult (seventeen weeks old)	
	Control	GA	Control	GA
Final body weight (g)	287.42±14.64	267.10±17.20*	521.17±26.26	500.71±28.12
Food intake (g/day)	18.02± 0.74	17.19± 1.26	23.65± 2.11	23.44± 1.38
Liver (whole g)	10.53± 2.87	11.53±1.01	15.47±2.06	14.49±1.61
(/100gB.W.)	3.64±0.89	4.31±0.23	2.96±0.25	2.89±0.22
Left testis (whole g)	1.23±0.20	0.90±0.10**	1.79±0.12	1.65±0.15
(/100gB.W.)	0.43±0.06	0.34±0.04*	0.34±0.03	0.33±0.04
Left epididymis (whole g)	0.24±0.04	0.25±0.03	0.74±0.04	0.66±0.07
(/100gB.W.)	0.08±0.01	0.09±0.01**	0.14±0.01	0.13±0.02
Right testis (whole g)	1.24±0.19	0.93±0.11**	1.80±0.15	1.67±0.21
(/100gB.W.)	0.43±0.06	0.35±0.05*	0.35±0.03	0.34±0.05
Right epididymis (whole g)	0.23±0.05	0.25±0.05	0.73±0.05	0.67±0.07
(/100gB.W.)	0.08±0.02	0.09±0.02	0.14±0.01	0.14±0.02
Kidney (whole g)	2.31±0.32	2.34±0.21	3.47±0.22	3.49±0.20
(/100gB.W.)	0.81±0.12	0.88±0.06	0.67±0.07	0.70±0.04
Spleen (whole g)	0.64±0.17	0.77±0.09	0.80±0.09	0.85±0.06
(/100gB.W.)	0.22±0.06	0.29±0.03	0.15±0.01	0.17±0.01
Perirenal fat (whole g)	4.32±0.74	3.61±0.98	10.37±2.03	10.26±2.51
(/100gB.W.)	1.51±0.28	1.34±0.33	1.99±0.37	2.05±0.49
Epidydimal fat (whole g)	3.06±0.88	2.66±0.67	8.28±1.28	6.77±0.84*
(/100gB.W.)	1.07±0.30	0.99±0.22	1.58±0.18	1.35±0.16*

Values are means±SD of 6-7rats in each group.
Significant differences between the Control group and GA group for each age were analyzed by two samples *t*-test (Dr. SPSS Ⅱ for windows).
Asterisks show significant difference; *:$p<0.05$, **:$p<0.01$

Table 3 Histological examinations of testis and epididymis in rats administered Garcinia

	Young								Adult							
	Control				Garcinia				Control				Garcinia			
	(+++)	(++)	(+)	(-)	(+++)	(++)	(+)	(-)	(+++)	(++)	(+)	(-)	(+++)	(++)	(+)	(-)
<testis>																
精細胞の変性	-	-	-	6/6	-	5/7	1/7	1/7	-	-	-	6/6	1/7	-	1/7	5/7
異常細胞の形成	-	-	-	6/6	1/7	4/7	2/7	-	-	-	-	6/6	-	-	-	7/7
＜epididymis＞																
精巣上体管腔の精子減少	-	-	-	6/6	6/7	-	1/7	-	-	-	-	6/6	-	-	1/7	6/7
精巣上体管腔の異常細胞	-	-	-	6/6	3/7	2/7	2/7	-	-	-	-	6/6	-	-	-	7/7

+++:marked ++:moderate +: mild -: negative

Table 4 Serum biochemical parameters of rats fed diet containing Garcinia

		Young		Adult	
Group		Control	GA	Control	GA
TP	g/dl	5.08± 0.31	5.39±0.13	5.88±0.08	5.84±0.11
Alb	g/dl	2.82± 0.18	2.98±0.15	2.88±0.04	2.94±0.10
A/G		1.24± 0.07	1.22±0.09	0.96±0.03	1.02±0.05**
BUN	mg/dl	5.67± 2.07	11.57±1.51***	11.50±1.38	14.29±1.70**
CRN	mg/dl	0.20± 0.02	0.17±0.02*	0.31±0.03	0.28±0.02
Glc	mg/dl	206.67± 28.66	214.57± 6.32	252.00±21.92	226.29±24.68
NEFA	mEq/l	0.91± 0.37	0.69± 0.10	0.83± 0.23	0.75± 0.10
PL	mg/dl	117.00± 24.96	143.86±19.70	135.00±12.00	120.29±20.29
TG	mg/dl	110.00± 70.03	60.00±16.61	110.50±48.20	54.71±22.26
TCho	mg/dl	56.33± 6.80	78.86±11.70**	71.00±10.20	69.00±13.99
AlP	IU/l	890.17±128.66	918.29±227.56	371.80± 79.37	367.71±40.66
ALT	IU/l	32.83± 6.01	26.71± 3.73*	42.67± 15.00	36.71±12.59
AST	IU/l	102.83± 22.27	83.86± 10.48	96.20± 22.66	102.00±20.14
ChE	IU/l	92.67± 33.75	87.71± 14.35	57.20± 11.01	67.57± 15.30
LAP	U	169.00± 19.36	154.29± 8.36	143.20± 17.43	141.86± 7.76
LDH	IU/l	330.50± 95.17	370.14±238.16	373.60±165.88	356.57± 93.70
Ketone body	μmol/l	509.00±219.67	388.14±234.32	387.20±149.60	574.14±358.12

Values are means±SD of 6-7rats in each group.
Significant differences between the Control group and GA group for each age were analyzed by two samples *t*-test (Dr. SPSS II for windows).
Asterisks show significant difference; *:$p<0.05$, **:$p<0.01$

Table 5 Hormone analysis of serum in rats administered Garcinia

	Young (seven weeks old)		Adult (seventeen weeks old)	
ng/ml	Control	GA	Control	GA
Inhibin-B	0.08±0.02	0.05±0.03***	0.06±0.03	0.06±0.02
FSH	7.20±1.50	12.40±3.10**	11.90±2.70	10.60±4.90
LH	5.00±1.10	4.60±0.70	5.40±1.20	5.20±0.70
Teststerone	0.71±0.52	2.00±1.20	2.90±1.20	3.60±3.10

Values are means±SD of 6-7rats in each group.
Significant differences between the Control group and GA group for each period were analyzed by two samples *t*-test (Dr. SPSS II for windows).
Asterisks show significant difference; *:$p<0.05$, **:$p<0.01$, ***:$p<0.001$

Table 6 Final body weight, food intake and each tissue weight

	For two weeks (ten weeks old)		For four weeks (twelve weeks old)	
	Control	GA	Control	GA
Final body weight (g)	233.50±16.92	224.38±14.97	261.00±17.97	235.25±15.37*
Food intake (g/day)	16.94±1.70	15.24±1.14	17.12±1.37	15.61±0.89
Liver (whole g)	8.03±0.93	8.10±0.58	8.44±0.85	7.89±0.91
(g/100g B.W.)	3.44±0.37	3.61±0.01	3.23±0.22	3.36±0.33
Left ovary (whole g)	0.067±0.011	0.065±0.004	0.065±0.013	0.064±0.009
(g/100g B.W.)	0.029±0.005	0.029±0.001	0.025±0.004	0.025±0.004
Right ovary (whole g)	0.058±0.009	0.058±0.007	0.064±0.009	0.060±0.007
(g/100g B.W.)	0.025±0.004	0.026±0.003	0.025±0.003	0.025±0.003
Uterus (whole g)	0.73±0.20	0.81±0.15	0.79±0.26	0.84±0.24
(g/100g B.W.)	0.32±0.01	0.36±0.05	0.31±0.12	0.36±0.12
Abdominal fat (whole g)	7.58±1.67	4.57±1.46*	11.42±2.11	6.47±1.63**
(g/100g B.W.)	3.22±0.52	2.02±0.56*	4.38±0.77	2.74±0.62**

Values are means±SD of 4-6rats in each group.
Significant differences between the Control group and GA group for each period were analyzed by two samples *t*-test (Dr. SPSS II for windows).
Asterisks show significant difference; *:$p<0.05$, **:$p<0.01$

Table 7 Histological examinations of ovary and uterus of rats administered Garcinia

	For two weeks								For four weeks							
	Control				Garcinia				Control				Garcinia			
	(+++)	(++)	(+)	(-)	(+++)	(++)	(+)	(-)	(+++)	(++)	(+)	(-)	(+++)	(++)	(+)	(-)
<卵巣>																
卵胞の減少	-	-	-	5/5	-	-	-	4/4	-	-	-	5/5	-	-	-	6/6
黄体の減少	-	-	-	5/5	-	-	1/4	3/4	-	-	-	5/5	-	-	1/6	5/6
卵巣の萎縮	-	-	-	5/5	-	-	1/4	3/4	-	-	-	5/5	-	-	1/6	5/6
<子宮>																
内腔の拡張									-	-	3/5	2/5	-	-	4/6	2/6
内膜の好酸球浸潤									-	-	3/5	2/5	-	-	5/6	1/6

+++:marked ++:moderate +: mild -: negative

Table 8 Serum biochemical parameters of rats fed diet containing Garcinia

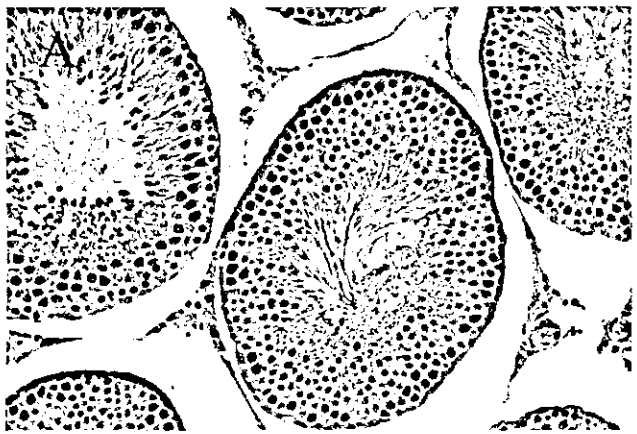
Group		For two weeks		For four weeks	
		Control	GA	Control	GA
TP	g/dl	5.98± 0.08	5.50± 0.32	6.08± 0.24	6.03± 0.49
Alb	g/dl	3.18± 0.11	2.88± 0.10	3.20± 0.22	3.05± 0.29
A/G		2.80± 0.10	2.63± 0.26	2.88± 0.04	2.98± 0.21
BUN	mg/dl	13.40± 1.14	12.50± 1.73	14.40± 0.89	14.83± 1.94
CRN	mg/dl	0.27± 0.03	0.25± 0.03	0.28± 0.02	0.29± 0.02
Glc	mg/dl	237.20± 23.29	227.20± 11.52	211.80± 17.91	209.00± 18.31
NEFA	mEq/l	0.48± 0.12	0.32± 0.04	0.39± 0.07	0.36± 0.07
PL	mg/dl	155.80± 18.21	140.00± 20.38	160.20± 31.12	139.33± 21.91
TG	mg/dl	37.80± 16.02	32.75± 10.97	53.80± 34.90	28.67± 10.23
TCho	mg/dl	76.80± 6.72	71.25± 8.46	76.40± 16.95	67.17± 11.86
AlP	IU/l	514.20±115.09	447.25± 94.61	296.40± 39.45	476.67± 81.52**
ALT	IU/l	24.60± 3.91	21.75± 2.36	23.80± 2.17	36.71± 12.59
AST	IU/l	58.60± 6.31	59.25± 6.65	55.20± 7.26	61.17± 10.01
ChE	IU/l	389.00± 98.35	297.75± 42.67	492.00±276.32	142.17± 4.79
LAP	U	134.60± 16.73	144.00± 11.34	126.40± 6.23	141.86± 7.76***
LDH	IU/l	107.00± 41.76	94.50± 51.20	117.00± 47.58	139.33± 66.10
Ketone body	μ mol/l	170.80± 57.01	182.75± 48.88	139.52± 25.36	174.87± 75.65

Values are means±SD of 4-6rats in each group.
Significant differences between the Control group and GA group for each period were analyzed by two samples *t*-test (Dr. SPSS II for windows).
Asterisks show significant difference; **:$p<0.01$, ***:$p<0.001$

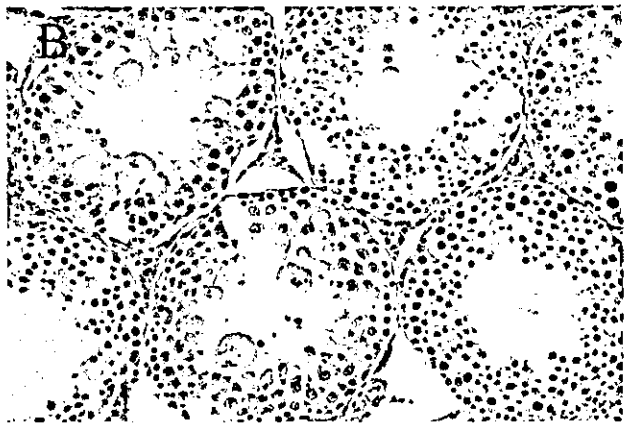
Table 9 Hormone analysis of serum in rats administered Garcinia

	For two weeks		For four weeks	
ng/ml	Control	GA	Control	GA
Estradiol(pg/mL)	49.80±11.45	40.75±17.52	39.60±20.84	46. 17±12.84
FSH	<3.62	< 4.28	4.46± 1.00	4.52± 1.79
LH	2.43±0.61	3.03± 1.41	3.26± 2.55	3.38± 1.48
Progesteron	20.72±9.16	15.93± 7.44	18.48±10.82	13.98± 7.94

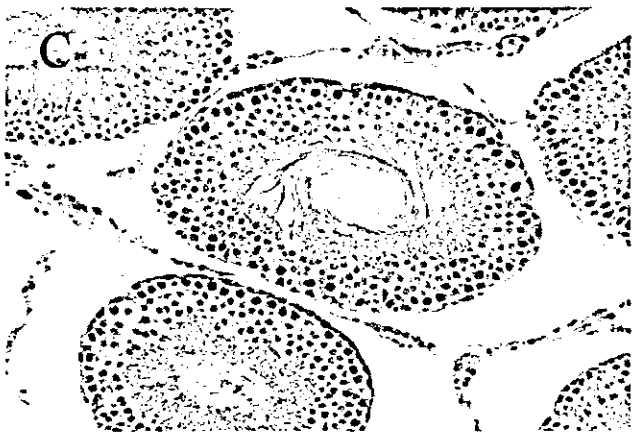
Values are means±SD of 4-6rats in each group.
Significant differences between the Control group and GA group for each period were analyzed by two samples *t*-test (Dr. SPSS II for windows).
Asterisks show significant difference; *:$p<0.05$, **:$p<0.01$, ***:$p<0.001$



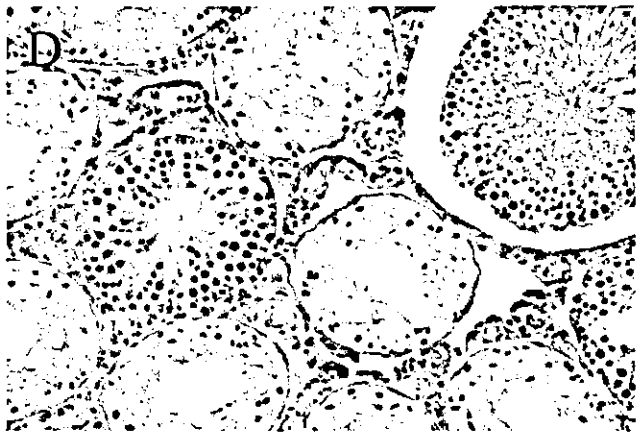
Degeneration of germ cell (−) Abnormal cell formation (−)



Degeneration of germ cell (++) Abnormal cell formation (++)



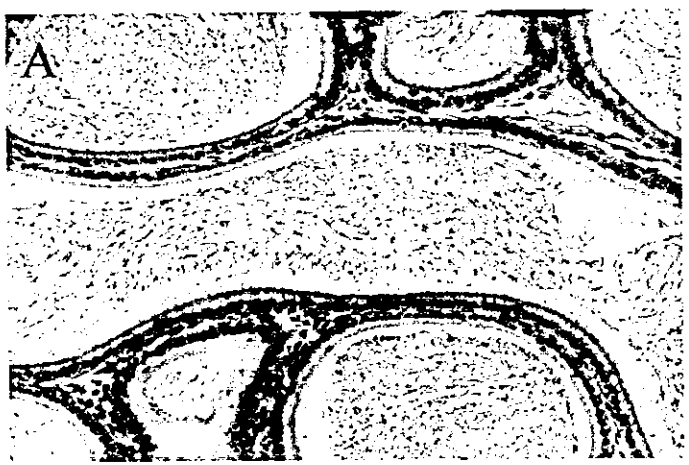
Degeneration of germ cell (−) Abnormal cell formation (−)



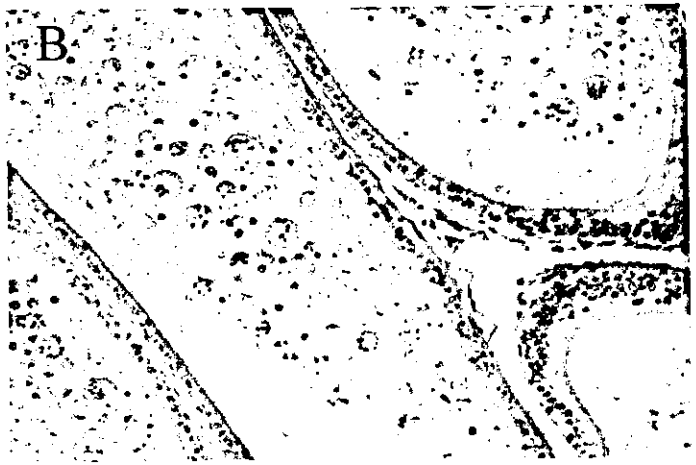
Degeneration of germ cell (+++)

Fig. 1 Histological examinations of testes of control (A) and GA (B) in young rats and those of control (C) and GA (D) in adult rats

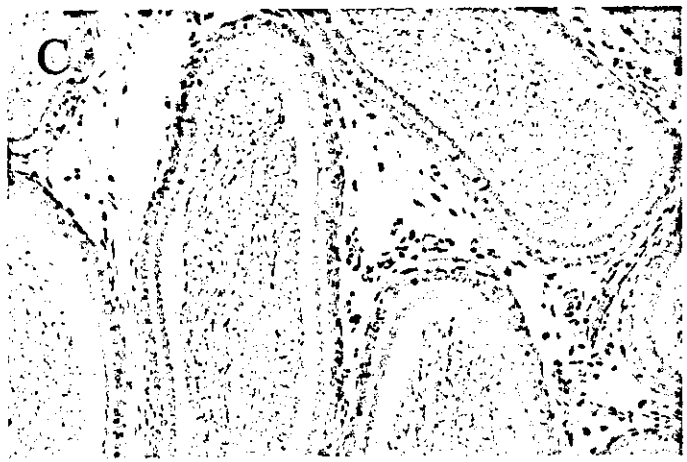
Four-week-old SD-IGS strain rats were fed control diet or Garcinia-containing diet for four weeks. Testes were fixed with 4% neutral-buffered formalin solution and stained with hematoxylin-eosin (magnification ×200).



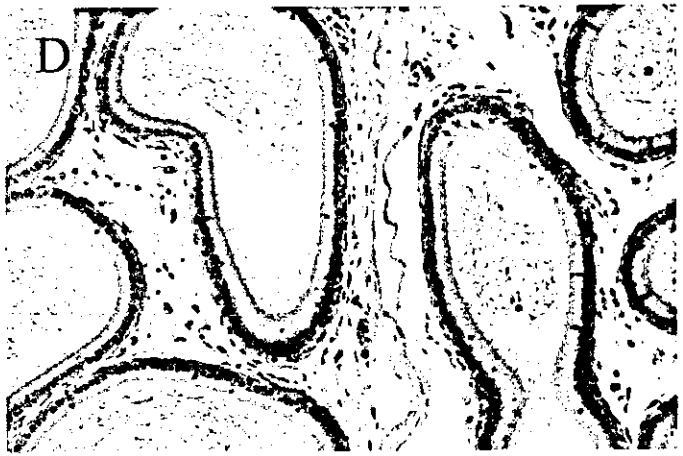
Decreased sperm in the duct (−) Abnormal cells in the duct (−)



Decreased sperm in the duct (+++) Abnormal cells in the duct (+++)



Decreased sperm in the duct (−) Abnormal cells in the duct (−)



Decreased sperm in the duct (+)

Fig. 2 Histological examinations of epididymides of control (A) and GA (B) in young rats and those of control (C) and GA (D) in adult rats

Four-week-old SD-IGS strain rats were fed control diet or Garcinia-containing diet for four weeks. Epididymides were fixed with 4% neutral-buffered formalin solution and stained with hematoxylin-eosin (magnification ×200).

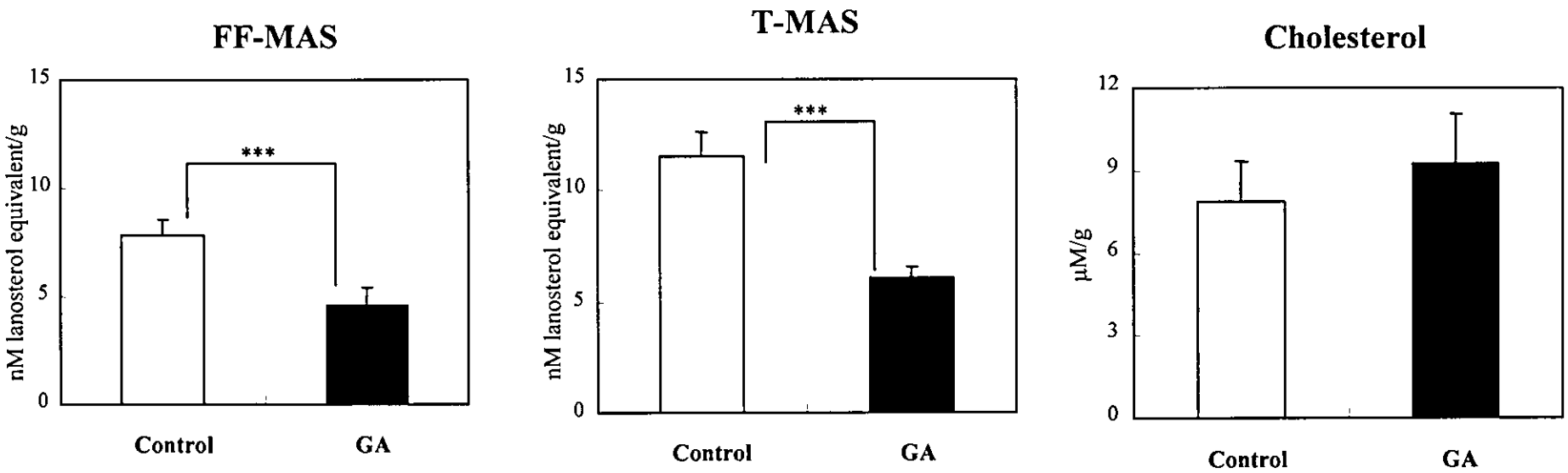


Fig. 3 Concentrations of FF-MAS, T-MAS and Cholesterol in young rat testis

Values are means±SD of 6-7rats in each group.
Significant differences between the Control group and GA group for young rats were analyzed by two samples t-test.
Asterisks show significant difference; ***:$p<0.001$

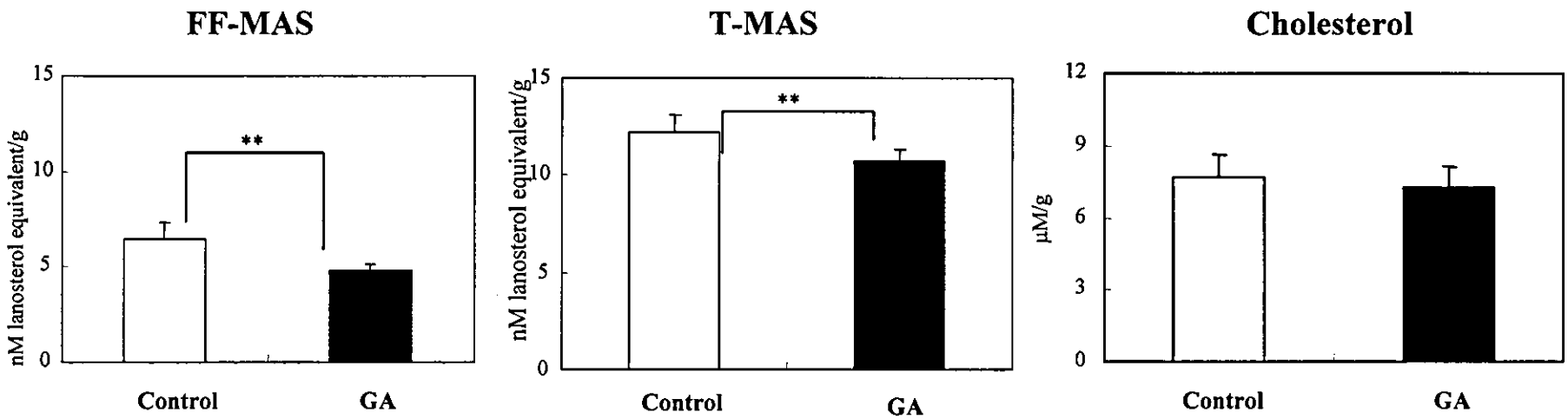


Fig. 4 Concentrations of FF-MAS, T-MAS and Cholesterol in adult rat testis

Values are means±SD of 6-7rats in each group.
Significant differences between the Control group and GA group for adult rats were analyzed by two samples t-test.
Asterisks show significant difference; **:$p<0.01$

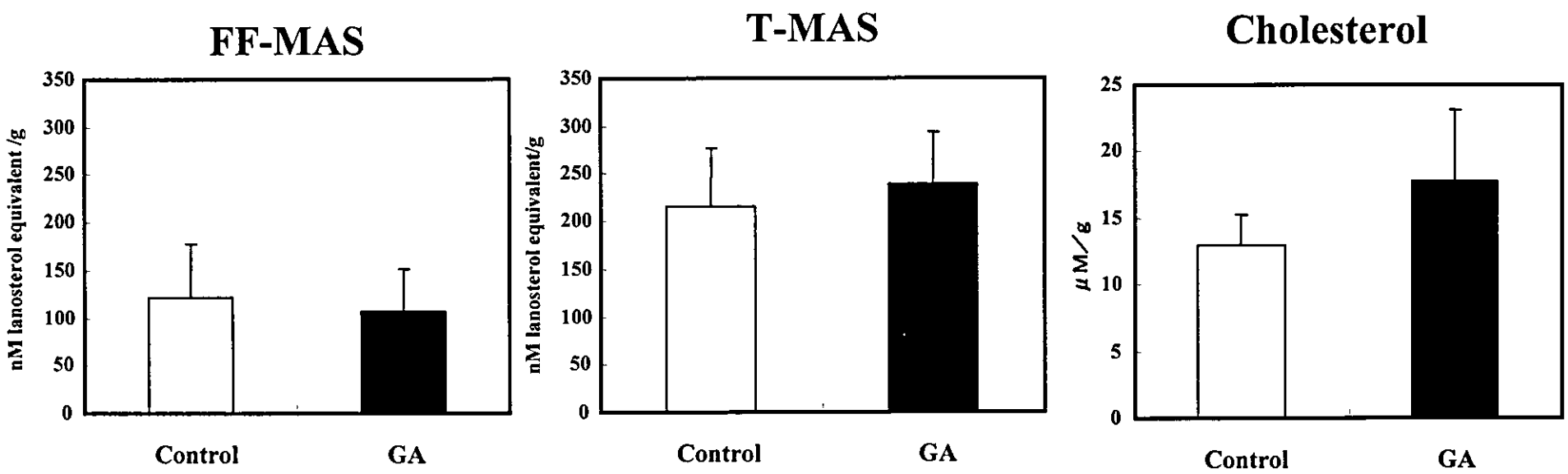


Fig. 5 Concentrations of FF-MAS, T-MAS and Cholesterol in rat ovary

Values are means±SD of 4-6rats in each group.
Significant differences between the Control group and GA group for two weeks period were analyzed by two samples t-test (Dr. SPSS II for windows).
Asterisks show significant difference; **:$p<0.01$

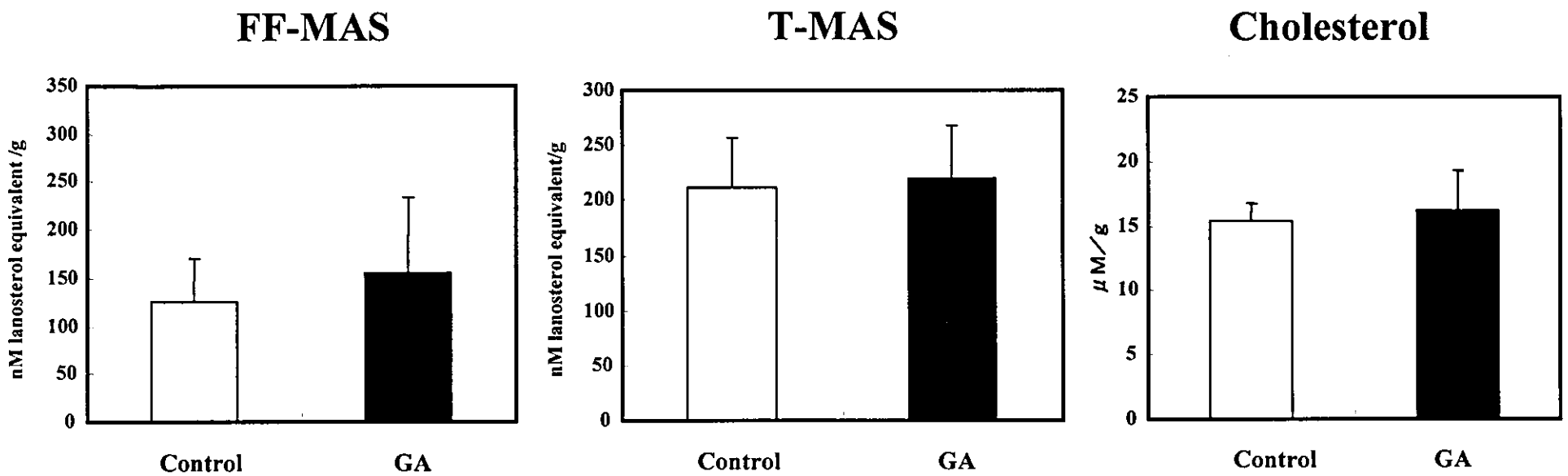


Fig. 6 Concentrations of FF-MAS, T-MAS and Cholesterol in rat ovary

Values are means±SD of 4-6rats in each group.
Significant differences between the Control group and GA group for four weeks period were analyzed by two samples t-test (Dr. SPSS Ⅱ for windows).
Asterisks show significant difference; **:$p<0.01$

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
該当なし							

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Sugiyama, T., Kubota, Y., Shinozuka, K., Yamada, S., Wu, J., Umegaki, K.	Ginkgo biloba extract modifies hypoglycemic action of tolbutamide via hepatic cytochrome P450 mediated mechanism in aged rats.	Life Sci.	75(9)	1113-1122	2004
李暁東、内田信也、山田浩、渡辺裕司、大橋京一、隠岐知美、大森由貴、丸山修治、梅垣敬三、山田静雄、木村良平	トルブタミドおよびミダゾラムの体内動態に対するイチョウ葉エキスの影響	臨床薬理	35(1)	208S	2004
嘉津山ひとみ、山下　毅、中村治雄、他.	特定保健用食品の組み合わせ摂取による有効性、安全性の検討II　－エコナ油とヘルシア緑茶の併用－	Prog. Med.	24	841-844	2004
宮島恵美子、山下　毅、中村治雄、他.	特定保健用食品の組み合わせ摂取の有効性、安全性の検討－大豆蛋白と高濃度カテキン茶の併用－	Prog. Med.	25	印刷中	2005
Kubo, K., Kiyose, C., Ogino, S. and Saito, M.	Suppressive Effect of Citrus Aurantium Against Body Fat Accumulation and Its Safety.	J. Clin. Biochem. Nutr.	36	11-17	2005
Saito, M., Ueno, M., Ogino, S., Kubo, K., Nagata, J. and Takeuchi, M.	High dose of Garcinia cambogia is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis.	Food Chem. Toxicol	43	411-419	2005

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Ginkgo biloba extract modifies hypoglycemic action of tolbutamide via hepatic cytochrome P450 mediated mechanism in aged rats

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Abstract

We examined hepatic cytochrome P450 (CYP)-mediated interactions between *Ginkgo biloba* extract (GBE) and tolbutamide, an oral anti-diabetic agent, in aged and young rats. Tolbutamide was orally given to rats with or without GBE treatment, and time-dependent changes in blood glucose were monitored. The basal activity of six CYP subtypes in liver was lower in the aged rats than in the young rats, while the inductions of these enzymes by 5 day pretreatment of 0.1% GBE diet were more in the aged rats. Further, the pretreatment of GBE significantly attenuated the hypoglycemic action of tolbutamide in the aged rats, corresponding well to the enhanced activity of (S)-warfarin 7-hydroxylase, which is responsible for CYP2C9 subtype, a major isoform metabolizing tolbutamide. In contrast, the simultaneous administration of GBE with tolbutamide potentiated the hypoglycemic action of this drug. The in vitro experiments revealed that GBE competitively inhibited the metabolism of tolbutamide by (S)-warfarin 7-hydroxylase in the rat liver microsomes. In the young rats, the 5 day pretreatment with GBE significantly attenuated the hypoglycemic action of tolbutamide, but a simultaneous treatment had little influence on the tolbutamide effect. In conclusion, the present study has shown that the simultaneous and continuous intake of GBE significantly affects the hypoglycemic action of tolbutamide, possibly via a hepatic CYP enzyme-mediated mechanism, particularly in the aged rats. Therefore, it is

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anticipated that the intake of GBE as a dietary supplement with therapeutic drugs should be cautious, particularly in elderly people.

Keywords: Ginkgo biloba extract; Cytochrome P450; Tolbutamide; Blood glucose; CYP2C9

Introduction

Ginkgo biloba extract (GBE) is a popular herbal medicines, and the leaf extract of *Ginkgo biloba* contains some functional flavonoids and terpenoids (Kleijnen and Knipschild, 1992a; Gruenwald et al., 2000). In some European countries, standardized GBE has been clinically used mostly for cerebral insufficiency, dementia, memory impairment, tinnitus and intermittent claudication (Kleijnen and Knipschild, 1992b; Le Bars et al., 1997; Ernst, 1999; Pittler and Ernst, 2000), while it is marketed as a dietary supplement in the United States and Japan.

GBE exerts many pharmacological effects, such as free radical-scavenging action and improvement of microcirculatory action (McKenna et al., 2001; De Smet, 2002; Ernst, 2002). On the other hand, GBE has been reported to have side effects such as headache, gastric symptoms, diarrhea, and allergic skin reactions, although the occasion of these side effects is rare (Cohen and Bartlik, 1998; De Smet, 2002; Ernst, 2002). Recently, adverse reactions of herbal remedies and potential herb-drug interactions have received a great deal of attention. As a well-known example, St John's Wort has been reported to induce hepatic CYP3A4 activity, thereby leading to the attenuation of efficacy of therapeutic drugs such as cyclosporin, indinavir, and digoxin (Durr et al., 2000; Roby et al., 2000). In the case of GBE, the occurrence of bleeding has been reported in patients who took GBE and anticoagulant drugs such as aspirin, rofecoxib, or warfarin at the same time (Vaes and Chyka, 2000; Izzo and Ernst, 2001). It is proposed that the mechanism of this adverse interaction of GBE with these drugs is due to the inhibitory effect of platelet activating factors by ginkgolide B, which is one of the active constituents of GBE (Braquet and Hosford, 1991). However, other interactions between GBE and drugs have not been fully elucidated.

In previous studies, we found that feeding GBE to rats markedly increased the concentration of hepatic cytochrome P450 (CYP), the expression of various CYP mRNA, and the enzyme activities in a dose- and time-dependent manner (Umegaki et al., 2000; Umegaki et al., 2002; Shinozuka et al., 2002). Moreover, we reported that pre-treatment with GBE decreased the hypotensive action by nicardipine, a calcium channel blocker, which is extensively metabolized by CYP3A type (Shinozuka et al., 2002). These findings indicate that similar interactions of GBE with other drugs through the mediation of CYPs might occur.

Because of its reported beneficial effects, many elderly people take GBE (Cohen and Bartlik, 1998; De Smet, 2002; Ernst, 2002). Generally, elderly people tend to suffer from hypertension and diabetes and thereby take some other drugs simultaneously with GBE. In particular, GBE has been shown to improve peripheral blood flow (Iliff and Auer, 1983) and is expected to prevent periphery necrosis and retinopathy in severe diabetes (Lanthony and Cosson, 1988; Doly et al., 1992). It is also reported that GBE inhibits the increment of blood glucose concentration in glucose-loaded diabetic rats (Rapin et al., 1997). Thus, the simultaneous intake of GBE and anti-diabetic agents might occur, particularly in elderly people.

The present study was undertaken to examine the hepatic CYP-mediated interactions of GBE with tolbutamide in aged and young rats. Tolbutamide is one of sulfonylureas applied for non-insulin-

dependent, type 2 diabetes, and its metabolism is dependent mainly upon the CYP2C9 activity in the liver (Tal, 1993; Proks et al., 2002; Lee et al., 2003). The effect of GBE intake on the hypoglycemia of tolbutamide in rats was evaluated with a 5 day pretreatment and simultaneous treatment of GBE.

Methods

Materials

A standardized powder form of *Ginkgo biloba* extract (GBE) was supplied by Tama Seikagaku-Kogyo Co., Tokyo. The GBE contained 24.9% flavonoids and 10.6% total terpene, which consisted of 2.9% ginkgolide A, 1.4% ginkgolide B, 2.1% ginkgolide C, and 4.2% bilobalide, and less than 1 ppm of ginkgolic acid. Tolbutamide, resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, testosterone, 6β-hydroxytestosterone, corticosterone, p-nitrophenol, 4-nitrocatechol, and 7-ethoxycoumarin were purchased from Sigma (St. Louis, MO, USA). (S)-Warfarin and 7-hydroxywarfarin were obtained from Ultrafine (Manchester, England). NADPH was obtained from Oriental Yeast (Tokyo, Japan). All other reagents were obtained from Wako Pure Chemical Ltd. (Osaka, Japan).

Animal experiment

Male Wistar aged (19 months old) and young (7 weeks old) rats obtained from Japan SLC (Shizuoka, Japan) were housed individually in stainless steel, wire-bottomed cages in a constant temperature room (23 ± 1 °C) under 12 hr light-dark cycle. Rats were divided into several groups with or without GBE treatment. GBE pretreated group was fed commercial rodent diet CE2 (Japan Clea, Tokyo) containing 0.1% GBE for 5 days. After the treatment, rats were orally administered tolbutamide (40 mg/kg). The simultaneous GBE treated group was given GBE (100 mg/kg body weight, p.o.) simultaneously with tolbutamide. After the tolbutamide administration, the blood was collected at predetermined time points and immediately blood glucose concentration was determined using a blood glucose detector Dexter Z II (Bayer Corp. Mishawaka, IN). After the treatment with or without GBE, the rat liver was removed and weighed, and a part of the liver was subjected to the enzyme assay.

All procedures were in accordance with National Institute of Health and Nutrition guidelines for the Care and Use of Laboratory Animals.

Analytical methods

Preparation of microsome and cytosolic fractions from the liver

The liver was rinsed with 0.9% NaCl solution, weighed and homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 10,000 × g at 4 °C for 30 min. The supernatant was further centrifuged at 105,000 × g at 4 °C for 60 min. The supernatant was used as the cytosolic fraction for the assay of glutathione S-transferase, the activity of which was determined by the method of Habig and Jakoby using 1-chloro-2,4-dinitrobenzene as a substrate (Habig and Jakoby, 1981). The pellet was washed once with 50 mM Tris-HCl buffer (pH 7.4) containing 0.25 M sucrose by

centrifugation at 105,000 × g at 4°C for 60 min, and the concentration and activities of CYP were analyzed.

Protein concentrations of microsome and cytosolic fractions were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Analysis of CYP enzyme activities

Cytochrome P450 content was quantified by the method of Omura and Sato (Omura and Sato, 1964). The activities of various CYP enzymes were determined by HPLC methods as reported previously (Umegaki et al., 2002). The subtypes of CYP enzymes examined and the corresponding CYPs were methoxyresorufin O-demethylase, CYP1A2; ethoxyresorufin O-deethylase, CYP1A1; pentoxyresorufin O-dealkylase, CYP2B; p-nitrophenol hydroxylase, CYP2E1; testosterone 6β-hydroxylase, CYP3A; and (S)-warfarin 7-hydroxylase, CYP2C9.

The interaction between GBE and tolbutamide toward (S)-warfarin hydroxylase activity in vitro were evaluated by adding these substances at various concentrations (GBE; 1.0–30 μg/mL, tolbutamide; 0.1–30 μg/mL) to the enzyme assay system ((S)-warfarin concentration; 2–16 μM).

Statistical analysis

Statistical analysis of the data was carried out using ANOVA followed by a post hoc test of Fisher's PLSD. These statistical analyses were performed using a computer program (Stat View 5.0, ASA Institute Inc, Cary, NC, USA).

Results

Induction of hepatic drug-metabolizing enzymes by GBE

Effects of 5 days of feeding of 0.1% GBE diet on the liver weight and hepatic drug metabolizing enzymes are shown in Table 1. The calculated intake of GBE was about 14 mg per day in both groups and

Table 1
Effects of 5 day pretreatment with GBE on the liver weight, cytochrome P450 content, and glutathione S-transferase activity in aged and young rats

	Aged rats		Young rats	
	Control	0.1% GBE	Control	0.1% GBE
Body weight (g)	433 ± 26	450 ± 18	189 ± 7	190 ± 6
Liver weight (% /Body weight)	2.76 ± 0.09	3.28 ± 0.06[a]	4.14 ± 0.19	4.12 ± 0.29
Cytochrome P450 content (nmol/mg protein)	0.731 ± 0.08	1.692 ± 0.33[a]	0.921 ± 0.05	1.749 ± 0.22[b]
Glutathione S-transferase (nmol/mg protein/min)	565 ± 197	1054 ± 217[a]	667 ± 104	934 ± 236[b]

Male Wistar aged (19 months old) and young (7 weeks old) rats received commercial rodent diet CE2 with or without 0.1% GBE for 5 days. Each value is the mean ± SD from six rats. Significant differences from each control level are indicated by [a]$P<0.01$ and [b]$P<0.05$.

was 32 mg (per kg body weight in a day) in the aged rats and 72 mg in the young rats. The liver weight in the aged rats but not in the young rats was significantly increased to 1.2-fold by the GBE treatment.

Significant increases in the cytochrome P450 content and glutathione S-transferase activity were also detected by the GBE treatment, both in the aged rats (2.3- and 1.9-fold, respectively) and young rats (1.9- and 1.4-fold, respectively). Activities of various CYP are shown in Table 2. The basal levels of the activities of six CYP subtypes were clearly lower in the aged rats than in the young rats. The activity of (s)-warfarin 7-hydroxylase, which is responsible for the metabolism of tolbutamide, in the aged rats was about 41% of that in the young rats. Both in the aged and young rats, the GBE pretreatment significantly increased all CYP enzymes, especially in pentoxyresorufin O-dealkylase and (s)-warfarin 7-hydroxylase activity. The induction ratios in both groups were apparently higher in the aged rats; the ratios in the young and aged rats were 13- and 42-fold for pentoxyresorufin O-dealkylase, respectively, and 2.1- and 4.7-fold for (s)-warfarin 7-hydroxylase activity, respectively.

Effects of GBE on the efficacy of tolbutamide

The time-dependent changes in the blood glucose concentration by tolbutamide administration in the aged rats are shown in Fig. 1. The baseline concentration of blood glucose was approximately 100 mg/dl. In the control group, the blood glucose concentration decreased to the minimum level (the reduction: 32.2 ± 2.4 mg/dl, n = 6) at 3 hr after oral administration of tolbutamide (40 mg/kg) and remained at the lower level until for least 7 hr. In the 0.1% GBE diet (5 days) pretreated group, the hypoglycemic action of tolbutamide was considerably attenuated when compared with that in the control group without GBE, and the statistical significance was detected at 2–5 and 7 hr. This result indicated that the pretreatment of GBE reduced the pharmacological action of tolbutamide. On the other hand, a simultaneous treatment of tolbutamide with GBE at a single dose (100 mg/kg body weight) significantly lowered (at 4–6 hr) the blood glucose concentrations in the aged rats, compared with the treatment of tolbutamide alone in the control group (Fig. 1). This result indicated that the simultaneous treatment of tolbutamide and GBE potentiated the hypoglycemic efficacy of tolbutamide. In the young rats, the pretreatment (5 days) of 0.1% GBE diet significantly attenuated the hypoglycemic action of tolbutamide as in the aged rats,

Table 2
Inductions of various hepatic CYP activities by 5 day pretreatment with GBE in aged and young rats

	Activity (pmol/mg protein/min)			
	Aged rats		Young rats	
	Control	0.1% GBE	Control	0.1% GBE
Ethoxyresorufin O-deethylase (CYP1A1)	13.5 ± 0.9	37.9 ± 1.4[a]	24.7 ± 1.5[b]	44.2 ± 1.6[a]
Methoxyresorufin O-demethylase (CYP1A2)	15.5 ± 1.1	33.2 ± 2.0[a]	27.6 ± 2.4[b]	41.7 ± 1.9[a]
Pentoxyresorufin O-dealkylase (CYP2B)	8.2 ± 0.3	348.4 ± 39.6[a]	35.5 ± 6.4	476.7 ± 23.6[a]
(s)-Warfarin 7-hydroxylase (CYP2C9)	2.6 ± 0.4	12.1 ± 0.8[a]	6.3 ± 0.4[b]	13.5 ± 1.2[a]
p-Nitrophenol hydroxylase (CYP2E1)	1453 ± 99	3580 ± 215[a]	1717 ± 76	4064 ± 170[a]
Testosterone 6β-hydroxylase (CYP3A)	218 ± 15	564 ± 42[a]	295 ± 30	739 ± 20[a]

Male Wistar aged (19 months old) and young (7 weeks old) rats received commercial rodent diet CE2 with or without 0.1% GBE for 5 days. Each value is the mean ± SD from six rats. Significant difference from each control level is indicated by [a]$P < 0.001$. Significant difference from control of aged rats is indicated by [b]$P < 0.01$.