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Research Paper

# Effects of *Ginkgo biloba* extract on the pharmacokinetics and pharmacodynamics of tolbutamide in protein-restricted rats

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## Abstract

**Objectives** Effects of repeated administration of *Ginkgo biloba* extract on pharmacokinetics and pharmacodynamics of tolbutamide were examined in rats fed a low-protein diet.
**Methods** Rats were given a low (7% casein) or control (20% casein) protein diet for 21 days and administered *Ginkgo biloba* extract (100 mg/kg per day) for the last 5 days. Tolbutamide was co-administered on the last day. Blood glucose and plasma tolbutamide concentrations were determined over the subsequent 12 h and the activity of hepatic cytochrome P450s were determined at 12 h after dosing.
**Key findings** There were significant decreases in body weight, the ratio of liver to body weight, and plasma albumin concentrations in rats on the low-protein diet compared with controls. The hypoglycaemic effect of tolbutamide was significantly greater and the concentration of the drug in plasma was higher in the former group. The repeated administration of *Ginkgo biloba* extract had little influence on the hypoglycaemic effect of tolbutamide, but tended to decrease the drug concentration in plasma of control rats, while it reduced significantly the hypoglycaemic action and plasma concentration of tolbutamide in the protein-restricted rats.
**Conclusions** The effects of *Ginkgo biloba* extract on the pharmacokinetics and pharmacodynamics of tolbutamide were significantly enhanced in rats on the low-protein diet.
**Keywords** *Ginkgo biloba* extract; herb–drug interaction; hypoglycaemic effect; protein restriction; tolbutamide

## Introduction

Elderly people are at high risk of drug–drug and food–drug interactions. The increased risk of such interactions might depend on several factors such as comorbidity, concomitant medication and nutritional status. The elderly often suffer from poor nutrition due to reduced food intake. Age-related changes in pharmacokinetics and pharmacodynamics may potentially amplify the risk of adverse events due to these interactions.[1,2] Thus, older people are more likely to be affected by drugs or dietary supplements. Additionally, elderly patients may have multiple diseases and, therefore, may take a variety of medications. Particular care is needed for medicines such as antidiabetic drugs and anticoagulants, augmentation of the pharmacological effects of which can be fatal.

Herbal remedies have received a great deal of attention as complementary and alternative medicines, and are used in many countries.[3] *Ginkgo biloba* extract is a very popular herbal medicine, used mostly by the elderly to treat dementia, depression, dizziness and tinnitus.[4] In addition, *Ginkgo biloba* extract might influence diabetic nephropathy.[5] Although little information is available on the adverse effects of herbal medicines, herb–drug interactions have been reported.[6,7] Previous studies found that *Ginkgo biloba* extract markedly increased the activity of some enzymes, and a clinical study has shown that *Ginkgo biloba* extract significantly decreased the area under the plasma concentration versus time curve ($AUC_{0-\infty}$) of tolbutamide and reduced its hypoglycaemic effect.[8–10] Similarly, pretreatment with *Ginkgo biloba* extract attenuated significantly the efficacy of tolbutamide, via the hepatic expression of the cytochrome P450 (CYP) enzyme, in aged rats.[11] In spite of the importance

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of elucidating food–drug interaction in the elderly, few experimental models are available to assess interactions in aged animals, whose use is both costly and time consuming.

It has been reported that dietary protein intake and serum albumin concentrations decline in the elderly, and that the rate of drug metabolism decreases with age.[12,13] Also, protein malnutrition has been reported to decrease significantly hepatic CYP levels in rats.[14] This study aimed to examine the effects of *Ginkgo biloba* extract on the pharmacokinetics and pharmacodynamics of tolbutamide in rats on a low-protein diet.

## Materials and Methods

### Materials

Powdered *Ginkgo biloba* extract was supplied by Tama Seikagaku-Kogyo Co. (Tokyo, Japan). It contained 24.9% flavonoid and 10.6% terpene trilactone, including 2.9% ginkgolide A, 1.4% ginkgolide B, 2.1% ginkgolide C, and 4.2% bilobalide. Tolbutamide, resorufin, ethoxyresorufin, methoxyresorufin, pentoxyresorufin, *S*-warfarin, 7-hydroxywarfarin, *p*-nitrophenol, 4-nitrocatecol, testosterone, 6$\beta$-testosterone, and chlorpropamide were purchased from Sigma-Aldrich (St Louis, MO, USA). NADPH was obtained from Oriental Yeast (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

### Animals and diets

Male Wistar rats (4-weeks old; Japan SLC Inc., Shizuoka, Japan) were kept in stainless steel wire-bottomed cages. They were housed two or three per cage in a room with a constant temperature of 24 ± 2°C and a 12 h light–dark cycle. After acclimatization for 3 days, the rats were given experimental diets containing 7% (low) or 20% (control) casein based on the AIN-93G formula for 21 days.[15,16] The composition of the diets is shown in Table 1. Food intake was measured daily. Both groups did not differ in dietary consumption. The initial body weight for the control diet group was 98 ± 1 g and for the low-protein diet group it was 95 ± 1 g (mean ± SE). Throughout the study rats were given free access to food and water.

**Table 1** Ingredients of the control (20%) diet and low (7%)-protein diet fed to rats

| Ingredient | Content (g/kg diet) | |
|---|---|---|
| | Control diet (20%) | Low-protein diet (7%) |
| Milk casein | 200 | 70 |
| Corn starch | 530 | 642 |
| Cellulose | 50 | 50 |
| Sucrose | 100 | 120 |
| L-Cystine | 3.00 | 1.05 |
| Choline bitartate | 2.5 | 2.5 |
| Soybean oil (no additives) | 70 | 70 |
| Vitamin mixture (AIN-93G) | 10 | 10 |
| Mineral mixture (AIN-93G) | 35 | 35 |
| Tertiary butylhydroquinone | 0.014 | 0.014 |

Rats fed the normal protein diet or the low-protein diet were orally given *Ginkgo biloba* extract (100 mg/kg per day) suspended in 0.5% carmellose (carboxymethylcellulose), or vehicle, for 5 days.[11] On the last day the rats were co-administered tolbutamide (40 mg/kg, suspended in soybean oil). Before and after tolbutamide administration, approximately 250 µl blood (total volume: approximately 2.5 ml) was collected from the tail vein. Blood glucose levels were measured with Accu-Chek Comfort (Roche, Basel, Switzerland). At 12 h after the administration of tolbutamide, rats were anaesthetized with pentobarbital and exsanguinated from the descending aorta with a heparinized syringe. The liver was removed and rinsed with 0.9% (w/v) NaCl. Plasma and liver samples were stored at −80°C before analysis.

All experiments were conducted in accordance with the guidelines of the Experimental Animal Ethics Committee of the University of Shizuoka.

### Determination of the concentration of tolbutamide in plasma

The preparation of plasma samples and the HPLC conditions were as reported by Bruce *et al*.[17] with some modifications. In brief, 10 µl chlorpropamide solution (100 µg/ml) as an internal standard and 50 µl 40% phosphoric acid were added to 50 µl plasma. The tubes were well vortexed and 2 ml chloroform was added as extract solvent. After 10 min of shaking, the sample was centrifuged at 2000*g* for 5 min, and 1.8 ml of the organic phase was dried under nitrogen at 40°C. The residue was dissolved with 100 µl mobile phase (31% solution of acetonitrile in 50 mM potassium phosphate buffer, pH 4.0) and 50 µl of the sample was subjected to HPLC. The HPLC system was composed of a pump (LC-10ADvp), an autosampler (SIL-10ADvp), and a UV detector (SPD-10Avp; Shimadzu, Kyoto, Japan). Tolbutamide and the internal standard were separated with an Inertsil ODS-3 column (3 µm, 4.6 × 150 mm; GL Sciences Inc. Tokyo, Japan) at a flow of 1.0 ml/min, and detected at 230 nm. Total recovery of tolbutamide in plasma was approximately 80% and the quantification limit was 0.5 µM. Intraday and interday precision data (coefficient of variance, CV, %) were approximately 3% and 10%, respectively.

### Measurement of cytochrome P450 activity in liver

The preparation of liver microsomes and analysis of CYP enzyme activity were performed by a method described previously.[9] The subtypes and corresponding activity of the CYP enzymes examined were: ethoxyresorufin *O*-deethylase, CYP1A1; methoxyresorufin *O*-demethylase, CYP1A2; pentoxyresorufin *O*-dealkylase, CYP2B; *S*-warfarin 7-hydroxylase, CYP2C; *p*-nitrophenol hydroxylase, CYP2E1; and testosterone 6$\beta$-hydroxylase, CYP3A. Protein concentrations were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

### Data analysis

The time-dependent changes in the plasma drug concentration and blood glucose concentration after the administration of tolbutamide from baseline (zero time) were plotted, and

absolute values were estimated. In fact, the area under the plasma concentration–time curve ($AUC_{0-12}$) and the area under the hypoglycaemic effect–time curve between 0 and 12 h ($AUEC_{0-12}$) were calculated by a noncompartmental analysis using WinNonlin Version 5.2 (Pharsight Corporation, Sunnyvale, CA, USA).

### Statistical analysis

The data are presented as the mean and standard error (SE) for each group. Statistical analyses were carried out by one-way analysis of variance with Dunnett's multiple comparison post hoc test, or by Student's *t*-test when two groups were tested. Differences of $P < 0.05$ were considered to be significant. These statistical tests were performed with Prism 4.03 (GraphPad Software, Inc., La Jolla, CA, USA).

## Results

### Effects of a low-protein diet on body weight and plasma albumin concentration in rats

The average daily food intake was 16.7 ± 0.3 and 20.1 ± 0.4 g for control diet-fed and low-protein diet-fed rats, respectively. Body weight, the liver/body weight ratio, and plasma albumin concentrations were significantly reduced by 15%, 21%, and 11%, respectively, in rats on the low-protein diet (Table 2).

### Effects of *Ginkgo biloba* extract treatment on the efficacy and plasma concentration of tolbutamide in rats on the low-protein diet

The time-dependent changes in the blood glucose concentration in rats after the tolbutamide administration are shown in Figure 1 and Table 3. The basal concentration of glucose in both control rats and low protein-diet rats was 128 ± 2 and 128 ± 3 mg/dl, respectively, thus it was not different between the groups. In control rats, the hypoglycaemic effect of tolbutamide was unaffected by the repeated administration of *Ginkgo biloba* extract (100 mg/kg per day) for 5 days (Figure 1a, Table 3). The hypoglycaemic effect of tolbutamide was considerably enhanced by the low-protein diet (718 ± 57 mg/dl·h) compared with the control diet (416 ± 9 mg/dl·h). Notably, the administration of *Ginkgo biloba* extract attenuated significantly the hypoglycaemic effect of tolbutamide in the rats on the restricted diet (Figure 1a). The area under the effect–time curve between 0 and 12 h ($AUEC_{0-12}$) of tolbutamide in rats fed the low-protein diet was significantly (31%) smaller in the *Ginkgo biloba* extract-treated group than a vehicle-treated group (Table 3).

The time-dependent changes in plasma tolbutamide concentrations were also examined. The concentrations increased in rats on the low-protein diet compared with those on the control diet, as shown by 1.5-fold higher $AUC_{0-12}$ values (Figure 1b, Table 3). Following the administration of *Ginkgo biloba* extract (100 mg/kg per day) for 5 days, plasma tolbutamide concentrations in control rats were decreased, but the change was insignificant (Figure 1b). However, tolbutamide concentrations in the low-protein diet-fed rats were significantly reduced (53%) (Figure 1b, Table 3).

### Induction of hepatic cytochrome P450 activity by *Ginkgo biloba* extract treatment

The repeated administration of *Ginkgo biloba* extract in both control and protein-restricted rats increased markedly (1.4–51-fold) the activity of each isoform of the CYP enzymes (Figure 2). The increase in the activity of pentoxyresorufin *O*-dealkylase and *S*-warfarin 7-hydroxylase responsible for CYP2B and CYP2C, respectively, was significantly greater in the group on the low-protein diet.

## Discussion

The health-promoting effects of foods have received a great deal of attention. In spite of the increasing use of herbal remedies, however, there is a lack of scientific evidence as to their safety. The elderly frequently use herbal remedies together with drugs.[18] Several studies have shown that the metabolism and clearance of drugs decrease in the elderly.[19,20] There are some indications of serious adverse events due to herb–drug interactions.[6,21] Thus, elderly people may be at high risk of food–drug interactions and an appropriate experimental model is needed to predict these. This study attempted to evaluate food–drug interactions in rats fed a low-protein diet, which may reflect some physiological features in the elderly.

Malnutrition is due to poor dietary intake and falls into the category of protein deficiency. Additionally, it has been reported that the weight of the liver decreases 40% by 80 years of age and that protein deficiency reduces plasma albumin concentrations and liver weight in rodents.[22–24] In this study, rats given a low-protein diet (7% protein) for 21 days exhibited significant decreases in body weight, the ratio of liver weight-to-body weight, and plasma albumin concentrations, indicating them to be a model of protein deficiency. The basal blood glucose concentration was unaffected by the low-protein diet. The delay of tolbutamide absorption might have been attributable to the nonfasted condition or the suspended state in oil. However, the hypoglycaemic effect of tolbutamide was significantly greater in low-protein diet fed rats than in the control group with higher plasma drug concentrations. The decrease in hepatic CYP activity in rats with

**Table 2** Body weight, liver weight, and the plasma albumin concentration in rats fed either a control diet or a low-protein diet

| Diet type | Initial body weight (g) | Body weight (g) | Liver weight/body weight (%) | Plasma albumin conc (g/dl) |
|---|---|---|---|---|
| Control | 98 ± 1 | 208 ± 2 | 3.63 ± 0.07 | 4.19 ± 0.07 |
| Low protein | 95 ± 1 | 176 ± 3*** | 2.88 ± 0.08*** | 3.73 ± 0.07*** |

Rats were fed a control diet or a low-protein diet for 21 days. Values are expressed as mean ± SE for 9–24 rats. ***$P < 0.001$ compared with control.

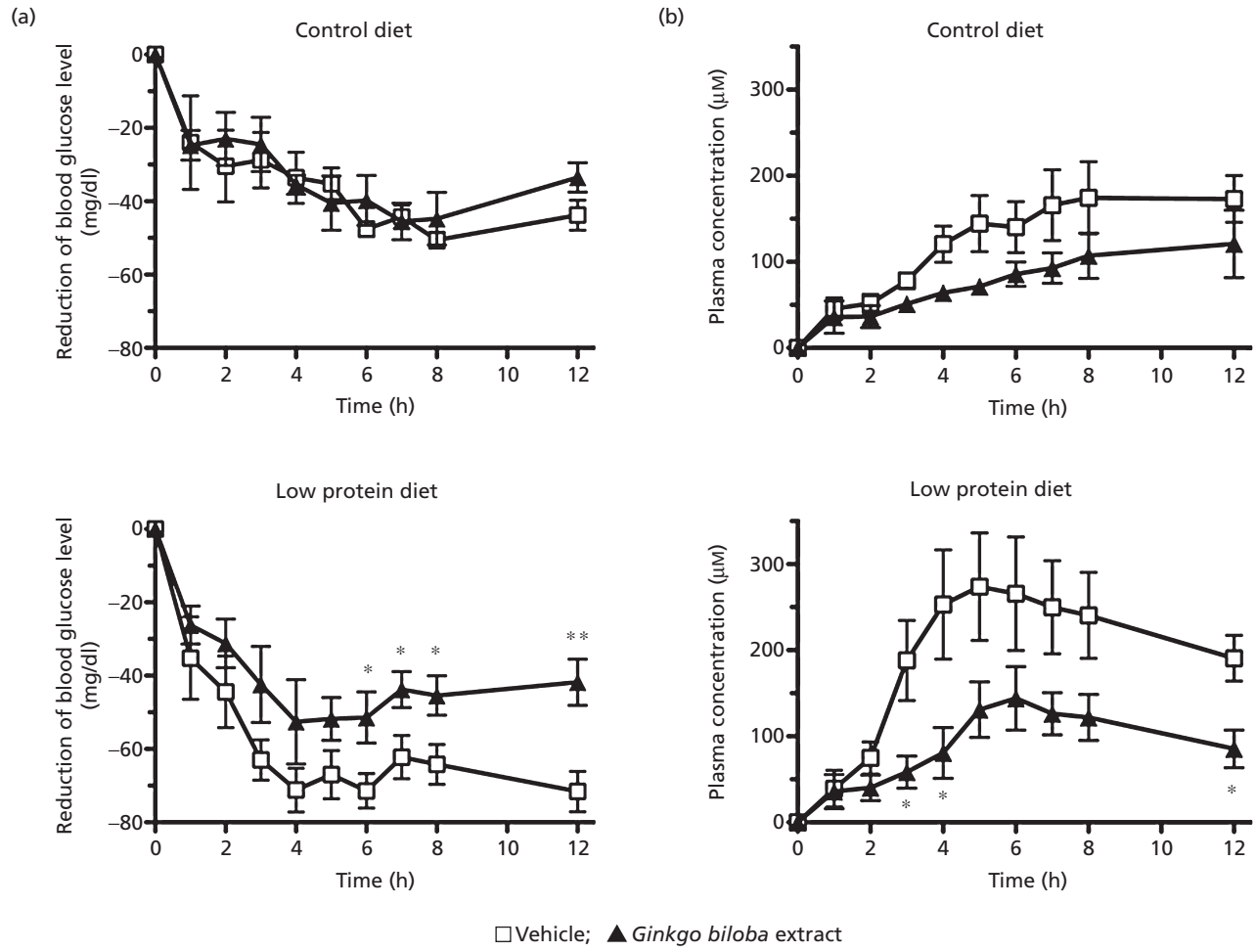


**Figure 1** Effects of repeated administration of *Ginkgo biloba* extract on the hypoglycaemic effect and plasma concentration of tolbutamide in rats fed a control diet or low-protein diet. (a) Hypoglycaemic effect of tolbutamide. (b) Plasma concentration of tolbutamide. Rats were fed the control diet or low-protein diet for 21 days and *Ginkgo biloba* extract (100 mg/kg per day) for the last 5 days, with tolbutamide (40 mg/kg) co-administered once on the final day of treatment. Each point represents the mean ± SE for four to six rats. $*P < 0.05$, $**P < 0.01$ compared with vehicle.

protein malnutrition has been reported previously.[14,25] It was reported also that a protein-restricted diet suppressed the expression of hepatic CYP2C11 by 80% in the rat liver and CYP1A2, CYP2E1 and CYP3A1/2 by 50–60%.[26] The basal activity of CYP2C was reported to be lower in aged rats than young rats.[11] In other words, protein restriction in rats may mimic the decreased levels of hepatic CYP enzymes in aged rats. Therefore, the observed augmentation of the hypoglycaemic effect of tolbutamide in low-protein diet-fed rats may have been attributable to the decreased disappearance via the decreased liver weight and decreased total activity of hepatic CYP.

The repeated administration of *Ginkgo biloba* extract had little effect on the hypoglycaemic effect of tolbutamide and only tended to decrease the plasma drug concentration in control rats. This agreed with previous reports in young rats.[11] Notably, however, repeated administration of *Ginkgo biloba* extract reduced significantly the hypoglycaemic action and plasma concentration of tolbutamide in the low-protein diet-fed rats. In other words, protein restriction in rats attenuated significantly the pharmacological effect of tolbutamide consistent with the marked decrease in the plasma drug concentration.

Tolbutamide is metabolized mainly by CYP2C9. Recent studies have suggested the occurrence of herb–drug interactions via the induction of hepatic CYP enzymes by *Ginkgo biloba* extract and the feeding of this extract to rats increased markedly the amount of hepatic CYP, the expression of various CYP mRNAs, and the activity of some enzymes.[8–10,27] In agreement with those results, repeated pretreatment with *Ginkgo biloba* extract induced markedly the expression of hepatic CYP enzymes in rats on the control diet and low-protein diet, and the increases in *S*-warfarin 7-hydroxylase (CYP2C) and pentoxyresorufin *O*-dealkylase (CYP2B) were significantly larger in the latter group (Figure 2). Our previous study showed that *Ginkgo biloba* extract produced a marked induction of hepatic drug-metabolizing enzymes, especially the CYP2B and CYP2C subtypes.[28] Consistent with these

**Table 3** Effects of repeated administration of *Ginkgo biloba* extract on the hypoglycaemic effect and plasma drug concentration of tolbutamide in rats fed either a control diet or a low-protein diet

| Diet type | Hypoglycaemic effect: $AUEC_{0-12}$ (mg/dl·h) | |
|---|---|---|
| | Vehicle | *Ginkgo biloba* extract |
| Control diet | 416 ± 9 | 412 ± 38 |
| Low protein diet | 718 ± 57 | 497 ± 67* |
| | Plasma concn: $AUC_{0-12}$ (μM·h) | |
| | Vehicle | *Ginkgo biloba* extract |
| Control diet | 1529 ± 273 | 947 ± 173 |
| Low protein diet | 2327 ± 464 | 1092 ± 202* |

Rats were fed either the control diet or low-protein diet for 21 days and *Ginkgo biloba* extract (100 mg/kg per day) for the last 5 days. Tolbutamide (40 mg/kg) was co-administered once on the last day of treatment. The area under the hypoglycaemic effect–time curve ($AUEC_{0-12}$) and area under the plasma concentration–time curve between 0 and 12 h ($AUC_{0-12}$) were estimated. Values are expressed as the mean ± SE for three to six rats. *$P < 0.05$ compared with vehicle.

results, among the CYP enzymes CYP2B and CYP2C were more sensitive to induction by *Ginkgo biloba* extract (Figure 2). Bilobalide was identified as a major constituent of *Ginkgo biloba* extract, involved in inducing hepatic CYP expression.[28] Thus, bilobalide may have remained in the plasma and liver for a longer period in the rats on the low-protein diet, contributing to the greater induction of CYP2B and CYP2C.

## Conclusions

The effects of *Ginkgo biloba* extract on the pharmacokinetics and pharmacodynamics of tolbutamide were significantly enhanced in rats on a low-protein diet.

## Declarations

### Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

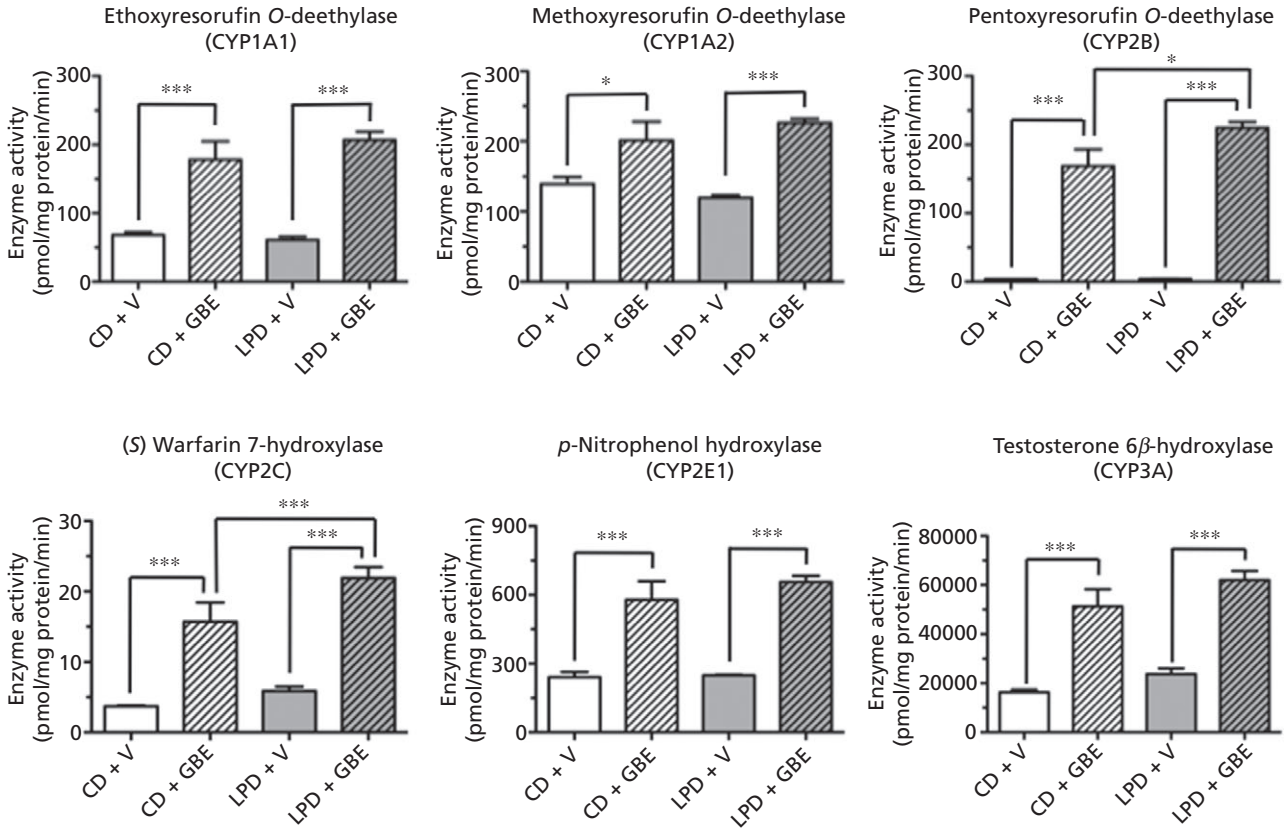


**Figure 2** Effects of repeated administration of *Ginkgo biloba* extract on activity of each isoform of hepatic cytochrome P450 from rats fed the control or low-protein diet. Rats were fed the control diet (CD) or low-protein diet (LPD) for 21 days and *Ginkgo biloba* extract (GBE; 100 mg/kg per day) or vehicle (V; 10 ml/kg) for the last 5 days. Tolbutamide (40 mg/kg) was co-administered once on the final day of treatment. The activity of each isoform of hepatic cytochrome P450 (CYP) was measured at 12 h after the *Ginkgo biloba* extract administration. Each column is expressed as the mean ± SE for four to six rats. *$P < 0.05$, ***$P < 0.001$.

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