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# Pharmacological Studies on *Puerariae flos* III: Protective Effects of Kakkalide on Ethanol-induced Lethality and Acute Hepatic Injury in Mice

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## Abstract

Kakkalide, one of the major isoflavonoid components of *Puerariae flos*, has been investigated for its effect on ethanol-induced intoxication and on hepatic injury, including hyperglycaemia, in mice.

Kakkalide reduced mortality associated with administration of ethanol. At doses of 100 and 200 mg $kg^{-1}$ the effect of kakkalide was significant. The same dose of kakkalide prevented increased serum glutamic oxaloacetic transaminase and glutamic pyruvic transaminase activity. At a dose of 200 mg $kg^{-1}$ it also counteracted ethanol-induced elevation of glucose levels.

These results suggest that kakkalide might be useful for counteracting the effects of alcohol and might be effective for treating hepatic injury.

In traditional Japanese and Chinese medicine *Puerariae flos* and its components, for example kakkakaiseito, are used in therapy to counteract problems associated with alcohol drinking. We have previously reported that the isoflavonoid fraction and the triterpenoid saponin fraction isolated from *Puerariae flos* have pharmacological effects, including effects on alcohol metabolism. Niiho et al (1989, 1990) reported that the isoflavonoid fraction suppressed the increase in the concentrations of blood ethanol, acetaldehyde, and ketones induced by ethanol administration, and that the isoflavonoid and triterpenoid saponin fractions improved both the unusual metabolism induced by ethanol and hepatic injuries induced by carbon tetrachloride or high-fat food. As a continuation of previous work we have studied the protective effect of kakkalide, one of the major isoflavonoid components of *Puerariae flos*, against toxicity, liver damage, and hyperglycaemia induced by ethanol.

## Materials and Methods

*Animals*

Male DDY strain mice, 30–38 g (SLC Japan, Shizuoka, Japan) were maintained in an air-conditioned room with lighting from 0700 to 1900. The room temperature (22 ± 2°C) and humidity (55 ± 10%) were controlled automatically. A laboratory pellet chow (Funabashi Farm, Chiba, Japan) and water were freely available.

*Materials*

Kakkalide (Fig. 1) was supplied by coworkers at Kumamoto University. This compound was suspended in saline solution.

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*Alcohol toxicity experiments*

DDY mice were fasted overnight and 25% ethanol in saline (7·5 g $kg^{-1}$) was injected intraperitoneally 30 min after kakkalide suspended in saline had been administered, also intraperitoneally. The control group received the vehicle only. The number of mice that had died 24 h after ethanol injection were counted.

*Ethanol-induced acute hepatic injury and hyperglycaemia*

DDY mice were fasted for 9–10 h and 25% ethanol in saline (5·0 g $kg^{-1}$) was injected intraperitoneally 30 min after kakkalide suspended in saline had been administered, also intraperitoneally. The control group received vehicle only. The mice were killed by cervical dislocation 15 h after ethanol administration and whole-blood samples were immediately withdrawn from the carotid artery. Serum obtained after centrifugation for 10 min at 12 000 rev $min^{-1}$ was assayed for glutamic oxaloacetic transaminase (GOT) activity, glutamic pyruvic transaminase (GPT) activity, and for glucose. GOT and GPT activity were measured by the method of Reitman & Frankel (1957). The measurement of serum glucose by the glucose oxidase method (Sharp 1972) was performed with a commercial test kit (Glucose B-test; Wako Pure Chemical, Osaka, Japan).

*Statistical analysis*

Data are presented as mean ± s.e.m. The statistical significance

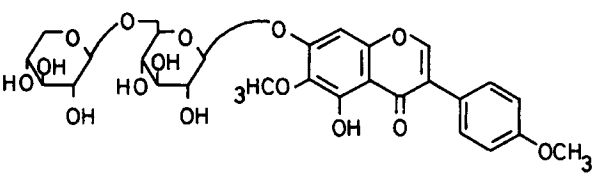


FIG. 1. The chemical structure of kakkalide.

of the results was evaluated by use of the $\chi^2$-test or the unpaired $t$-test.

## Results

*Alcohol toxicity*

The mortality 24 h after administration of 25% ethanol was 92·5%. The data in Table 1 show that mortality was reduced when the mice were treated with kakkalide 30 min before administration of the ethanol. Kakkalide at 100 mg $kg^{-1}$ or 200 mg $kg^{-1}$ reduced mortality significantly, to 70% and 65%, respectively.

*Ethanol-induced acute hepatic injury and hyperglycaemia*

Increased serum GOT and GPT activity were significantly reduced in mice given kakkalide at a dose of 100 mg $kg^{-1}$ or 200 mg $kg^{-1}$, respectively, compared with the values obtained for the control group (Table 2). Treatment of mice with 25% ethanol also resulted in an increase in serum glucose; this was significantly suppressed by administration of kakkalide at a dose of 200 mg $kg^{-1}$ whereas kakkalide at a dose of 100 mg $kg^{-1}$ had no significant effect (Table 3).

## Discussion

According to the Chinese Drugs Dictionary (1985) *Puerariae flos* is used in traditional medicine to counteract the effects of drinking. Kurihara & Kikuchi (1975) reported the isolation of a new isoflavone glycoside, kakkalide (ca 0·34% dry weight) from *Puerariae flos*.

The purpose of this investigation was to study the preventive effects of kakkalide on ethanol-induced mortality and hepatic injury in mice. The results indicate that kakkalide has anti-lethal and hepatoprotective properties; in mice it reduced mortality induced by ethanol administration and reduced the ethanol-induced increase in serum GOT and GPT activity. Suda et al (1986) and Fujiwara et al (1988) have suggested that the lethal effect of ethanol might be derived from hepatic toxicity or sympathomimetic activity caused by release of catecholamines.

Kakkalide reduced mortality 24 h after administration of 25% ethanol; at a dose of 100 mg $kg^{-1}$ and 200 mg $kg^{-1}$ kakkalide had a particularly pronounced anti-lethal effect. These results show that kakkalide is effective as a treatment for alcohol intoxication.

Kakkalide suppressed the increase in serum GOT and GPT activity induced by ethanol administration. Estimation of the serum activity of GOT and GPT is one of the most widely used means of measuring hepatocellular injury (Chenoweth & Hake 1962). Administration of ethanol in mice induced an increase in serum GOT and GPT as a parameter of liver injury. Many studies have found that serum catecholamine content seems to play an important role in ethanol-induced hepatic injury. Klingman & Haag (1958) indicated that injection of a large dose of ethanol resulted in an increase in serum catecholamine content and Yoshihara et al (1986) noted that ethanol-induced hyperadrenalaemia caused hepatic injury characterized by suppression of hepatic blood flow. Our results, together with the findings above suggest that ethanol-induced mortality and hepatic injury in mice are a result of inhibition of hepatic blood flow induced by hyperadrenalaemia. Treatment of mice with kakkalide seemed to preserve the integrity of liver-cell membranes, as evidenced by the reduction in the ethanol-induced increase in GOT and GPT activity. Furthermore, kakkalide suppressed the increase in serum glucose content induced by administration of ethanol. Svendsen et al (1978) reported that in man ethanol could increase blood glucose after a short fast or no fast, and Topping et al (1979) reported that ethanol infusion raised

Table 1. Mortality in mice 24 h after ethanol injection.

| Treatment | Number of dead mice/ total number of mice | Mortality (%) |
|---|---|---|
| Control | 37/40 | 92·5 |
| Kakkalide 12·5 mg $kg^{-1}$ | 17/20 | 85·0 |
| Kakkalide 25 mg $kg^{-1}$ | 16/20 | 80·0 |
| Kakkalide 50 mg $kg^{-1}$ | 15/20 | 75·0 |
| Kakkalide 100 mg $kg^{-1}$ | 14/20 | 70·0* |
| Kakkalide 200 mg $kg^{-1}$ | 13/20 | 65·0** |

*$P < 0.05$; **$P < 0.01$ (unpaired $t$-test), significantly different from control group.

Table 3. Effect of kakkalide on serum glucose levels 15 h after ethanol injection in mice.

| Treatment | n | Glucose (mg $dL^{-1}$) |
|---|---|---|
| Normal | 12 | 71·04 ± 3·49 |
| Control | 12 | 104·52 ± 14·52 |
| Kakkalide 100 mg $kg^{-1}$ | 12 | 75·58 ± 3·29 |
| Kakkalide 200 mg $kg^{-1}$ | 12 | 66·71 ± 3·07** |

*$P < 0.05$; **$P < 0.01$ (unpaired $t$-test), significantly different from control group.

Table 2. Effect of kakkalide on serum glutamic oxaloacetic transaminase activity and glutamic pyruvic transaminase activity 15 h after ethanol injection in mice.

| Treatment | n | Glutamic oxaloacetic transaminase (mUnits $mL^{-1}$) | Glutamic pyruvic transaminase (mUnits $mL^{-1}$) |
|---|---|---|---|
| Normal | 12 | 155·18 ± 7·24* | 18·05 ± 2·21** |
| Control | 12 | 178·93 ± 6·84 | 88·80 ± 10·36 |
| Kakkalide 100 mg $kg^{-1}$ | 12 | 152·00 ± 8·61* | 50·31 ± 8·50** |
| Kakkalide 200 mg $kg^{-1}$ | 12 | 152·28 ± 6·33** | 48·69 ± 6·43** |

*$P < 0.05$; **$P < 0.01$ (unpaired $t$-test), significantly different from control group.

perfusate glucose concentrations and caused an increase in hepatic glucose output. Nihira (1982) has indicated that ethanol-induced hyperglycaemia or increased hepatic glucose output might be a result of glyconeogenesis caused by hyperadrenalaemia. Our observation that increased serum glucose levels can be measured 15 h after ethanol administration is equivalent to their finding. We found that pre-treatment with kakkalide provides protection against glucose metabolism damage by reducing the high glucose levels induced by ethanol. In particular, kakkalide at a dose of 200 mg $kg^{-1}$ was shown protect against hepatic injury and glucose metabolism damage induced by ethanol administration. This supports our previous report that ethanol-induced hyperglycaemia was inhibited by a methanolic extract isolated from *Puerariae flos.*

This study has investigated the effects of kakkalide on alcohol intoxication and ethanol-induced hepatic injury. Kakkalide protected against ethanol-induced mortality and it is assumed that the administration of kakkalide to mice prevented inhibition both of hepatic blood flow and of glyconeogenesis associated with the increased of serum catecholamine content induced by ethanol. Although a satisfactory explanation of the effect observed is not yet apparent, it is conceivable that the glyconeogenetic system in the liver might be restored by administration of kakkalide.

This study clearly demonstrates that kakkalide protects against the effects of alcohol metabolism and counteracts the effect of alcohol consumption. The results also suggest that *Puerariae flos* is effective for treating hepatic disease, which is interesting in light of the observation that kakkalide might be a useful drug for inhibiting the effects of alcohol intoxication. Further detailed investigation, including study of pharmacological mechanisms, will be necessary to complete this study.

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