Effects of isoflavones on alcohol pharmacokinetics and alcohol-drinking behavior in rats[1–3]

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ABSTRACT Puerarin, daidzin, and daidzein are 3 major isoflavonoid compounds isolated from *Pueraria lobata*, an edible vine used widely in China for various medicinal purposes. We studied the antiinebriation and the antidipsotropic effects of these antioxidants in rats. Daidzin and daidzein shortened alcohol-induced sleep time (loss of righting reflex) in rats that were given ethanol intragastrically but not in those given ethanol intraperitoneally. When daidzin was given to animals intragastrically with the ethanol solution, the blood alcohol concentration (BAC) was found to peak later and be lower than in control rats that were given only the ethanol solution. BACs also receded more slowly if daidzin was fed to the animals. None of the 3 isoflavonoid compounds administered orally affected liver alcohol dehydrogenase or aldehyde dehydrogenase activities, as was reported for intraperitoneal administration. Further experiments indicated that the suppression of the BAC by daidzin was due mainly to delay of stomach emptying. All 3 compounds suppressed voluntary alcohol consumption in alcohol-preferring rats. The decrease in alcohol consumption was accompanied by an increase in water intake, so that the total volume of liquid consumed daily remained unchanged. Daily food consumption and body weight gain were not affected. Alcohol preference returned to baseline levels after the isoflavonoids were discontinued. We postulate that the suppression of alcohol reinforcement produced by these compounds is mediated centrally in the brain reward pathway.
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KEY WORDS Isoflavone, alcohol, daidzin, daidzein, puerarin, P rat, hamster, herbal medicine

INTRODUCTION

Alcohol abuse and alcoholism are serious and costly problems in our society. Thus, the development of antialcoholism agents would be highly desirable. A few traditional medicines have been used as remedies for ethanol intoxication in the Orient for hundreds of years, yet the effective components of these remedies and their antiinebriation mechanism remain mostly unknown. During the past few years, the antiintoxicant properties of the extract from an edible vine, *Pueraria lobata*, have drawn a considerable amount of attention. Puerarin, daidzin, and daidzein are 3 of the major isoflavonoid compounds isolated from the extract of *P. lobata* (**Figure 1**). Research in several laboratories studying different rodent models has produced a few reports describing the effects of these isoflavonoid compounds on alcohol pharmacokinetics and drinking behavior. This review summarizes and compares the findings of these reports. It is hoped that these studies will stimulate further investigations of these compounds and other constituents of edible plants that may exert similar or more potent actions.

EFFECT OF DAIDZIN ON SLEEP TIME INDUCED BY ETHANOL

Rats, when given a large dose of ethanol intragastrically or a lower dose intraperitoneally quickly lose the righting reflex when placed on their back. The time between the loss and the regain of righting reflex is called sleep time. Early tests performed at the Henan Medical Institute, Zhengzhou, Henan, People's Republic of China, showed that feeding the crude extract from *P. lobata* as well as purified daidzin or daidzein, 2 of the major components in the herbal extract, shortened the sleep time induced by alcohol in mice (M Kai et al, unpublished observations, 1988).

It was hypothesized then that daidzin and daidzein might accelerate alcohol clearance from blood. To test this hypothesis, we carried out similar experiments in Wistar rats (1). Ethanol (7 g/kg body wt) given intragastrically to Wistar rats deprived of food and water for 24 h induced sleep time lasting for nearly 4.5 h. When the same amount of ethanol was mixed with daidzin (30 mg/kg body wt) and given intragastrically to the rats as a bolus, the sleep time was reduced to 3 h (**Table 1**). However, oral administration of daidzin did not significantly shorten the sleep time if ethanol (2 g/kg body wt) was injected intraperitoneally into rats, whether the compound was given 60 min before or immediately before the ethanol injection. Our data therefore indicated the absence of an effect on ethanol elimination rate by daidzin in vivo. Consistent with this conclusion, we found that none of the 3 isoflavones (daidzin, daidzein, and puerarin) affected the activities of alcohol-

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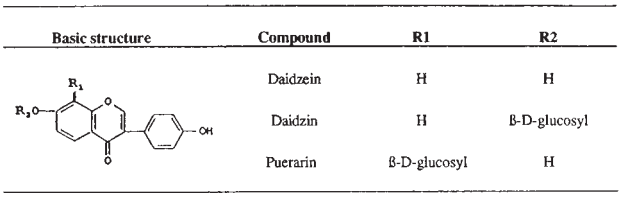

FIGURE 1. Chemical structures of daidzein, daidzin, and puerarin.

metabolizing enzymes (ie, alcohol dehydrogenase and aldehyde dehydrogenase) in rat livers whether the compounds were given by intubation acutely or chronically (1, 2).

Blood samples were taken at intervals after the animals were given ethanol intragastrically for measurement of the blood alcohol concentration (BAC). The BAC rose quickly and peaked in 30 min in control food-deprived rats and was maintained at the peak level for 30 min before declining almost linearly to ≈40% of the peak concentration 5 h after alcohol ingestion (**Figure 2**). In contrast, the BAC of rats that had been given daidzin and alcohol concurrently rose more slowly and peaked only at 90 min. The peak BAC of the daidzin-treated rats was significantly lower than that of the control group. Furthermore, the BAC of rats treated with daidzin fell at a slower rate than in the untreated control animals and thus was higher 5 h after alcohol feeding. Similarly, daidzin also suppressed and delayed the peaking of the BAC after ethanol ingestion in fed animals. Chronic daidzin administration also affected alcohol pharmacokinetics, but the effect was less pronounced than when the compound was administered concurrently with ethanol. The possibility that this effect of daidzin might be due to delayed stomach emptying was investigated by feeding rats [^{14}C] polyethylene glycol ([^{14}C]PEG) mixed with ethanol because PEG cannot be absorbed by the gastrointestinal tract. Animals that received daidzin retained 23% more [^{14}C]PEG as well as ethanol in the stomach than did the control rats 30 min after feeding (1).

Isoflavones are potent antioxidants. Therefore, 2 other unrelated antioxidants, vitamin E and thiotic acid, were tested for their effect on the righting reflex of rats given alcohol. Results showed that similarly to daidzin, both chemicals significantly shortened sleep time and delayed as well as decreased the peak BAC (1). However, the effective amounts of vitamin E and thiotic acid were 5–10 times higher than of daidzin.

Our study thus showed that daidzin is effective in countering alcohol intoxication by suppressing the BAC through delayed stomach emptying, but not by enhancing the clearance of ethanol in circulation. Furthermore, the effect of daidzin may in part be due to its antioxidant activity.

EFFECTS OF ORAL ISOFLAVONES ON ALCOHOL CONSUMPTION

To test for the antidipsotropic effects of the isoflavonoid compounds, we used a pharmacogenetic rat model for alcoholism, the genetically selected, alcohol-preferring P line of rats (P rats), developed through selective breeding for high alcohol consumption (Alcohol Research Center, Indiana University School of Medicine, Indianapolis) (3). The P rats attain intoxicating blood ethanol concentrations with 2-bottle, free-choice drinking of water and an aqueous ethanol solution (10%, by vol) and develop tolerance and physical dependence. P rats will press a bar to obtain ethanol either orally or by intragastric infusion.

The P rats were individually caged for the experiments, which lasted 21 d. Crystalline isoflavonoid compounds were weighed and mixed with 1 g of moist, ground, laboratory rat food. The mixture was reshaped into pellets and air dried. Similar pellets without the isoflavonoid compounds were prepared in the same manner (control pellets). On day 1, food baskets were removed at the beginning of the light cycle; 12 h later a 1-g control pellet was offered to each rat just before the beginning of the dark cycle. As soon as the animal consumed the food pellet (usually within 2 min), the basket containing a preweighed quantity of normal rat food was placed in the cage. Animals were then allowed free access to food and free choice drinking of water and 10% ethanol during the entire dark cycle. At the beginning of the next light cycle, the amounts of food, water, and alcohol consumed were recorded; the food basket was again removed; and the water and 10% ethanol bottles were refilled. This protocol was repeated for 7 d to familiarize the animals with the feeding routine and to obtain baseline readings for each animal. On day 8, test animals were given test pellets while control animals continued to receive control pellets. On day 14, all animals were switched back to control pellets for an additional 7 d to test for the reversibility of the drug effect. None of the compounds tested (daidzin, daidzein, and puerarin, up to 300 $mg \cdot kg^{-1} \cdot d^{-1}$) showed apparent toxic effects, as the animals gained weight normally during the entire 21 d.

All P rats consumed from 6 to 9 g $ethanol \cdot kg^{-1} \cdot d^{-1}$ before the treatment (**Figure 3**A). Daily alcohol consumption was dramatically reduced after animals received daidzein and daidzin. The effect of both daidzein and daidzin was apparent within 1 d after treatment but reached nadir after 2 d. The decrease in alcohol consumption in the test animals was accompanied by a reciprocal increase in water intake (Figure 3B) so that the volume of total daily liquid intake by the test animals was almost the same as that by control animals (Figure 3C). Thus, the decrease in alcohol intake is not the result of thirst reduction by the test compound. The effects of these compounds on ethanol and water intakes were reversible. When the treatments were discontinued, both alcohol and water consumption returned to control levels within 2 d. Daily food consumption was not affected (Figure 3D). Puerarin was also effective in suppressing alcohol consumption by the P rats (2), but amounts <30 $mg \cdot kg^{-1} \cdot d^{-1}$ were not effective. When the compounds were given orally at 100 $mg \cdot kg^{-1} \cdot d^{-1}$, their relative efficacy was daidzein > daidzin > puerarin.

The mechanism for the suppression of alcohol intake by these isoflavones is not known. Although delayed gastric emptying may, in part, be mediating the decreased drinking of ethanol, the

TABLE 1
Effect of daidzen on sleep time[1]

Treatment	Sleep time
	min
Ethanol (7 g/kg body wt) given intragastrically (n = 8)	
No daidzin, ethanol only	267 ± 22
Daidzin with ethanol concurrently	177 ± 31[2]
Ethanol (2 g/kg body wt) given intraperitoneally (n = 3)	
No daidzin, ethanol only	74 ± 6
Daidzin followed by ethanol immediately	65 ± 6
Daidzin followed by ethanol 30 min later	67 ± 3
Daidzin followed by ethanol 60 min later	66 ± 4

[1] $\bar{x}$ ± SD. Daidzin (30 mg/kg body wt) was given intragastrically. Reproduced from reference 1 with permission.

[2] Significantly different from no daidzin, $P < 0.05$.

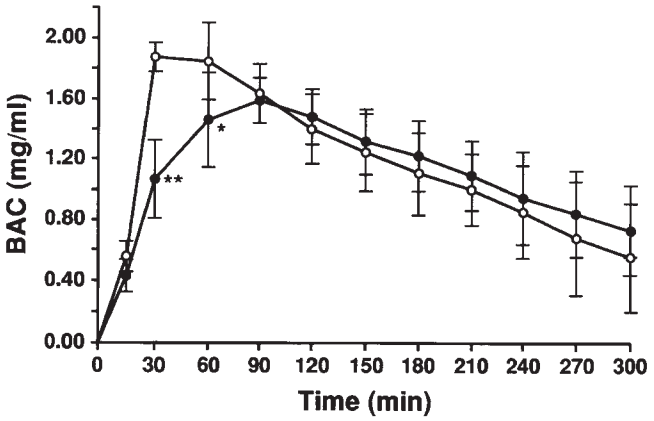

FIGURE 2. Mean (±SD) effect of daidzin on the blood alcohol concentration (BAC) in fasted rats. Food and water were removed from rats for 24 h before the experiments. Rats were given ethanol at 3 g/kg body weight in 40% alcohol solution with (●) or without (○) daidzin at 30 mg/kg body wt intragastrically. Blood samples were obtained from the tail at the indicated time after administration of alcohol. Each point represents the mean values of 6 rats. Brackets indicate the SDs of the group. *,**Significantly different from control: $^{*}P < 0.05$, $^{**}P < 0.01$. Reproduced from reference 1 with permission.

compensatory increase in water consumption and the lack of effect on food consumption mitigate this possibility. The reversibility of the effect and the similar delay periods for the suppression and the recovery of alcohol preference do not favor the hypothesis of conditioned taste aversion for alcohol by these isoflavones. Thus, the effect may be more ethanol specific, such as altering taste factors or acting on the central nervous system to suppress or stimulate ethanol-responsive neurotransmitter-modulator systems in the reward pathway.

EFFECTS OF INTRAPERITONEALLY ADMINISTERED ISOFLAVONES ON ALCOHOL CONSUMPTION

The effect of the isoflavones given by intraperitoneal injection on alcohol intake has been tested in P rats and in Fawn-Hooded rats. Fawn-Hooded rats have naturally low concentrations of serotonin and prefer alcohol. Overstreet et al (4) reported that NPI-028, a semicrude extract from several different medicinal herbs including *P. lobata*, injected acutely into both strains of rats decreased alcohol intake in a continuous-access schedule (24-h access to food, water, and alcohol in a 2-bottle choice fashion) as well as in a limited-access schedule (animals had continuous access to food and water but access to alcohol for only 1 h each day). Intraperitoneal injection of purified puerarin also decreased alcohol intake by both rat strains (4). Curiously, rats given isoflavones by intraperitoneal injection did not show compensatory increases in water consumption. The reversibility of the alcohol intake suppression was not examined in this study, but a preliminary study seemed to indicate that NPI-028 did not induce taste aversion in rats (5). Furthermore, a single injection of NPI-028 into the P rats 20 min before intraperitoneal administration of ethanol had no significant effect on the BAC clearance rate (4). The authors therefore concluded that NPI-028 may act by reducing the reward pathway of alcohol.

The antidipsotropic effect of isoflavones from *P. lobata* has also been investigated in Syrian golden hamsters. Syrian golden hamsters are desert-adapted rodents that highly prefer ethanol under the 2-bottle, free-choice regimen (6). The animals, however, metabolize alcohol quickly and have a relatively low BAC after consuming large amounts of alcohol. Additionally, they tolerate a large amount of alcohol and do not show signs of alcohol withdrawal, and therefore are seldom used in alcohol research. Keung and Vallee (7) reported that when Syrian golden hamsters were injected intraperitoneally with a large dose (1.5

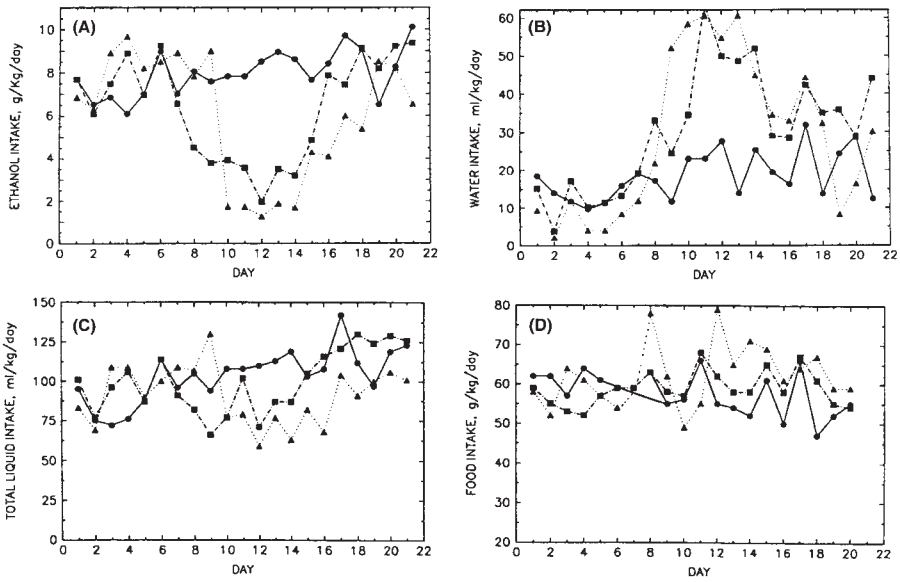

FIGURE 3. Time course of the effects of daidzein and daidzin. P rats were given normal rat food from day 1 through day 7. Rats in test groups then ingested daidzein or daidzin incorporated into food pellets at a dose of 100 mg · kg^{-1} · d^{-1} from day 8 through day 14 (inclusive) while the control group remained on normal food. All rats were fed normal laboratory food without the isoflavonoid compound from day 15 through the end of the experiment, day 21. Animals were allowed free choice of water or 10% ethanol for the entire 21 d. Each point represents mean values of 4 rats. ●, control group; ▲, daidzein group; ■, daidzin group. Reproduced from reference 2 with permission.

$g \cdot kg^{-1} \cdot d^{-1}$) of the crude extract from *Radix puerariae*, their free-choice alcohol intake was suppressed by 50%. Purified daidzin (150 $mg \cdot kg^{-1} \cdot d^{-1}$) and daidzein (230 $mg \cdot kg^{-1} \cdot d^{-1}$) were similarly effective whereas puerarin was not. The antidipsotropic effect seen in the hamsters was reversible (7). The doses used in their study were much higher than those used for the P rat study (2). Because dose-response curves were not reported, it is not known whether lower doses would be effective in hamsters. The major difference between the P rats and the hamsters was that the decrease in alcohol consumption by rats given isoflavones orally was compensated for by increased water consumption. As a result, daily liquid intake by the animals remained the same as for rats that received no isoflavones. Because water intake was not affected in hamsters injected with the isoflavonoid compounds, there was a net decrease ($\geq$30%) in daily liquid consumption. It is not clear whether the decrease in liquid intake is a characteristic of desert animals or is due to the intraperitoneal injection of isoflavones, because the decrease of alcohol consumption in rats brought on by injection of NPI-028 was also not compensated for by an increase in water intake (4).

A study by Keung and Vallee (8) showed that daidzin was a potent inhibitor for mitochondrial low-K_m aldehyde dehydrogenase in vitro, and Keung (9) showed that daidzein inhibited alcohol dehydrogenase. Therefore, they postulated at first that these isoflavones may deter alcohol drinking by interfering with alcohol metabolism in Syrian golden hamsters. However, more recently these investigators showed that neither blood ethanol nor acetaldehyde concentrations were affected in hamsters injected with daidzein (10). These latest results, therefore, agree with our finding that isoflavones do not affect alcohol metabolizing enzymes in vivo. Furthermore, they suggest that the alcohol-avoiding action by isoflavones is different from that of disulfiram, which is thought to involve a peripheral action (ie, elevation of blood acetaldehyde concentrations).

SUMMARY

Recent studies in animal models have shown that isoflavonoid compounds from *P. lobata* interfere with ethanol action. Oral ingestion of daidzin and daidzein shortens the sleep time induced by intragastric but not intraperitoneal administration. The antiintoxication effect may be due, in part, to the antioxidant activity of isoflavones. Although some isoflavonoid compounds extracted from *P. lobata* are potent inhibitors of alcohol and aldehyde dehydrogenase activities in vitro, they have no effect on these enzymes in vivo. The lower BAC seen when ethanol is given orally with daidzin is most likely due to delayed stomach emptying. Isoflavonoid compounds from *P. lobata* suppress alcohol intake in alcohol-preferring rodents, including P rats, Fawn-Hooded rats, and Syrian golden hamsters. The antidipsotropic effect of these compounds is not mediated by alterations of blood alcohol or acetaldehyde concentrations, and is reversible. Current data suggest that the effect is probably not mediated by conditioned taste aversion but may involve some steps in the reward pathway of the central nervous system. Further experiments will be needed to delineate these mechanisms.

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