

## RAPID COMMUNICATION

# Daidzin Decreases Ethanol Consumption in Rats

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In a previous study, daidzin, a constituent of an ancient Chinese herbal treatment for alcoholism, decreased home-cage ethanol consumption in laboratory Syrian golden hamsters. The present study tested the generality of daidzin's antidipsotropic effects. Rats served as subjects in a two-lever choice procedure. At one lever, responses earned 10% ethanol, flavored with saccharin. At the other lever, responses earned an isocaloric starch solution. Daidzin decreased both ethanol and starch consumption, but the decreases in ethanol intake were larger. Changes in consumption were dose dependent, and differences in ethanol and food consumption increased slightly (but significantly) as dose increased. Daidzin produced a similar pattern of decreases in lever pressing. In baseline, there was an approximately equal distribution of responses between the two levers; at the highest daidzin dose, the relative number of responses at the ethanol lever decreased to 30%. These results replicate and extend earlier findings, and they encourage further research on daidzin's capacity to decrease ethanol consumption.

**Key Words:** Daidzin, Ethanol Consumption, Ethanol Preference, Kudzu Plant, Rats.

FOR THE LAST several decades, investigators of the pharmacology of alcohol have sought a drug that would selectively decrease the desire for alcohol.<sup>1-4</sup> Recently, Keung and Vallee<sup>5-7</sup> approached this problem in a seemingly novel way. They started with an herbal mixture that has been used by traditional Chinese physicians for more than a millennium for the treatment of alcohol abuse.<sup>6,8</sup> One of the ingredients is *Radix puerariae*, the root of the kudzu plant (*Pueraria lobata*). Keung and Vallee<sup>5,6</sup> isolated a component of *P. lobata* that decreases ethanol consumption in Syrian golden hamsters and inhibits mitochondrial aldehyde dehydrogenase, an enzyme involved in the metabolism of alcohol in humans.<sup>9</sup> The active component is an isoflavone known as daidzin. The laboratory findings appear to be consistent with the discoveries and long-held traditions of Chinese physicians, thereby suggesting that

daidzin or one or more of its congeners and/or metabolites might be effective in treating excessive alcohol consumption and/or abuse.

The hamster study constituted the first published account of daidzin's behavioral effects. It is not known whether daidzin will decrease ethanol consumption in other species or whether daidzin's antidipsotropic properties were specific to ethanol. As a first step toward answering these questions, we tested the effects of daidzin on ethanol consumption in rats, using a procedure that provides an isocaloric food control.

The experimental chamber was equipped with two levers. Presses at one earned 10% ethanol flavored with saccharin; presses at the other earned an isocaloric starch solution (Polycose). In previous experiments in which these or similar conditions were used,<sup>10,11</sup> rats drank large amounts of ethanol (e.g., 3 g/kg) in relatively short periods of time (30–45 min), and it was possible to independently manipulate ethanol consumption and food consumption. For example, pre-session meals of sucrose and chow decreased sucrose consumption but not ethanol consumption, whereas pre-session meals of ethanol did just the reverse.<sup>10</sup> Thus, the procedure has advantages for research aimed at identifying the unique biological and behavioral features of ethanol: it promotes high and rapid rates of drinking, provides an isocaloric food control, and establishes conditions under which the motivation to consume ethanol can be dissociated from that of consuming food. A recent experiment, conducted in another laboratory, obtained similar results using a sweetened ethanol versus sucrose choice procedure.<sup>12</sup>

## METHODS

### Subjects

The subjects were seven male Wistar rats, 50 days old, with free-feeding weights of approximately 220 g at the initiation of training. During training and a preliminary pilot study, the rats were fed 10 g of chow after daily experimental sessions. They gradually gained weight, stabilizing at approximately 365 g. During the course of the study reported here, the target weights were 365 g for six of the rats, and 350 g for the seventh. These weights were maintained by a daily diet of 8 to 12 g of chow, depending on the rat, provided after the session. The energy value of this diet plus ethanol and Polycose consumption during experimental sessions was about 50 kcal/day.

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### Apparatus

The experiments were conducted in seven standard operant chambers (MED Associates: 28 × 20.5 × 26 cm). Two levers (5 cm wide) were inserted into the front wall, 7 cm above the floor and 1 cm from each side. Just below each lever (2 cm) was an opening into which a 0.1-ml dipper cup could be raised. The dippers, which were operated by lever presses, sat in troughs which were filled with solutions of Polycose or ethanol. Lever presses of 0.25 N or more closed a switch, and these switch closures on occasion operated the dipper (as described in "Experimental Procedure"). Experimental events were arranged and recorded with an IBM compatible personal computer that used MED-PC software.<sup>13</sup>

### Pre-experimental Induction of Ethanol Consumption

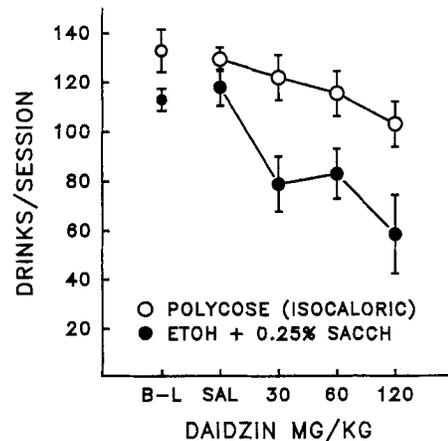
Before the experiments, the rats were induced to drink ethanol. The goal was to establish high rates of consumption. Initially, one dipper provided water, and the other dipper provided 10% sucrose mixed with ethanol. Ethanol concentration was gradually increased from 2.5 to 25% (v/v) in 2.5 and 5% steps, with 5 to 10 sessions at each concentration. (The absence of an increasing or decreasing trend in response rates for three consecutive sessions was the criterion for changing ethanol concentration.) During this phase of training, median ethanol consumption levels varied from 2.50 to 4.75 g/kg/30-min session.

In the next phase of training, the rats were introduced to the ethanol and isocaloric food condition. The ethanol mixture was manipulated first. Its concentration was reduced from 25 to 10%, and then the concentration of the sucrose vehicle was gradually reduced from 10 to 0%. When sucrose was at a concentration of 2.5%, saccharin was added (in amounts that produced an 0.25% solution). Next, the water in the second trough was replaced by a mixture of Polycose and water. The initial Polycose concentration was 2.5%, and because it provides approximately 3.8 kcal/g, the final concentration was 14.8%. This was accomplished in small steps of approximately 2.5% increases. Thus, at the end of training, one dipper offered a mixture of 10% ethanol flavored with 0.25% saccharin, and the other dipper offered a mixture of 14.8% Polycose. Both solutions provided 0.56 kcal/ml.

The training and an initial pilot study (not reported) lasted 239 sessions. Thus, the results presented here were preceded by a 40-week period during which the animals consumed large amounts of ethanol daily (typically about 2.5 to 3.0 g/kg/30-min session). New ethanol solutions were mixed each day and kept in sealed flasks until the start of the experimental session.

### Experimental Procedure

The experimental procedure was based on one that has proven a reliable and sensitive assay for measuring biological and environmental induced changes in preference.<sup>14-16</sup> Left lever presses intermittently operated the left dipper (making the drinking cup available), and right lever presses intermittently operated the right dipper. For each lever and dipper pair, a variable-interval timer determined when responses were effective. While a timer was running, responses at its respective lever had no programmed consequences. However, once the timer elapsed, the next response at its lever operated the associated dipper. The dipper remained available for 3 sec, long enough for the rat to empty the cup (0.1 ml). This was followed by a 1.5-sec blackout, and then the timer that had elapsed was restarted with a new interval (drawn at random), and the process started anew. For each lever, the list of intervals approximated a Poisson distribution,<sup>17</sup> with a mean of 5 sec. The shortest interval was 1 sec and the longest was 16 sec. Importantly, the two variable-interval timers ran independently of one another. For example, while the subject was responding on the left lever, the right timer could elapse and set up a reinforcer for a right lever response. However, as is usually the case in concurrent interval schedule experiments, a brief delay (1.5 sec) had to elapse before a primed reinforcer could be delivered. That is, switches were never reinforced. This contingency eliminates adventitious switching.<sup>14</sup>



**Fig. 1.** On the x axis is dose and on the y axis is drinks/session, averaged across the seven subjects. Each daidzin dose was administered on three occasions and in ascending order. Saline was administered five times through the course of the study. Baseline (B-L) was defined as the session just preceding the saline (SAL) injection. Error bars indicate standard errors of the mean (between rat variation).

### Drug Injections

Injections were given either once or twice a week, with at least two nondrug days separating injections. Each dose, 30, 60, and 120 mg/kg, was given on three different occasions, and in ascending order, e.g., three 30 mg/kg doses before the first 60 mg/kg dose, to assess possible order effects. In addition, five saline injections were given at irregular intervals throughout the study. Injections occurred 60 min before the session.

### Blood Ethanol Measurements

Blood was sampled from the tail. To increase the likelihood that tail samples would reflect central concentrations, the tail was first warmed in a heating pad set at about 40°C.<sup>18</sup> A widely used enzymatic assay (Sigma) served to estimate blood ethanol concentration. The blood was taken right after the completion of the 30-min session, and the session was one in which the rats were not injected with daidzin or saline.

## RESULTS

Figure 1 summarizes the relationship between daidzin injections and Polycose and sweetened ethanol consumption. On the x axis is dose. On the y axis is average number of drinks/session (collapsing across subjects and treatment conditions: five saline doses, five baseline sessions, and three administrations of each daidzin dose). "Baseline" was defined as the session just before a session in which saline was injected. Because there were five saline injections, there were also five baseline sessions.

Saline and baseline sessions occurred throughout the study. The small standard errors indicate that the between-subject and within-subject variation was not large and that there were no large secular trends. For example, the average between-session standard error for ethanol preference was less than 2% in saline sessions.

Daidzin decreased both flavored ethanol and polycose consumption [ $F(3,18) = 12.78, p < 0.001$ ]. Post-hoc polynomial contrast tests revealed a linear trend for drug dose, but no higher order trends. At each dose, decreases in

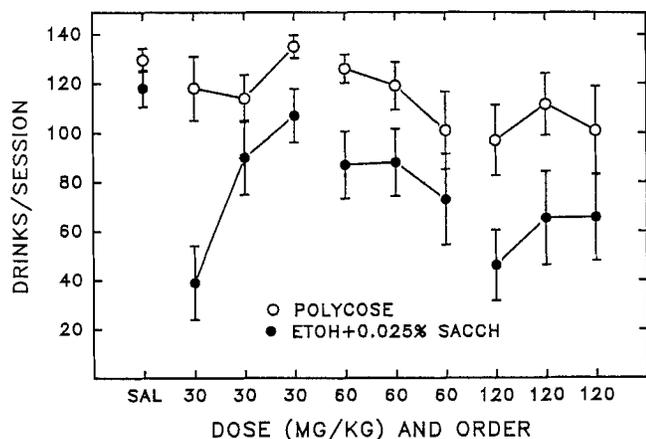


Fig. 2. On the x axis is dose and order of treatment. On the y axis is the number of drinks/session, averaged across the seven rats. Error bars indicate standard errors of the mean.

ethanol consumption were larger than decreases in Polycose consumption [ $F(1,6) = 6.5, p < 0.05$ ], that is, there was a significant "type-of-drink" effect. Moreover, as dose increased, the difference between ethanol and Polycose consumption increased (dose by type-of-drink interaction [ $F(3,18) = 4.96, p < 0.02$ ]), which, according to polynomial contrast tests, had significant linear and cubic components. Thus, daidzin decreases ethanol consumption more than it decreases Polycose consumption.

Figure 2 provides a dose-by-dose history of the study. These data were analyzed in two ways: an analysis of variance for the entire data set (dose, order of injection for a given dose, and type drink), and an analysis of variance across sessions for each drink at each of the three doses. The overall analysis shows essentially the same results as the averaged data: there was a significant dose effect [ $F(3,18) = 12.87, p < 0.001$ ], with larger doses producing larger decreases in responding [ $F(1,6) = 29.94, p < 0.002$ ]; the decreases in ethanol consumption were significantly larger than the decreases in Polycose consumption [ $F(1,6) = 6.5, p < 0.05$ ]; and the differences between sweetened ethanol and Polycose consumption increased as a function of dose (dose by type-of-drink interaction [ $F(1,6) = 6.1, p < 0.05$ ]). Order of injection for a given dose was significant for ethanol at the 30 mg/kg dose [ $F(2,12) = 8.3, p < 0.01$ ], but for no other dose. Thus, drug effects depended on the type of drink and dose level, and after the first administration of daidzin, drug effects did not vary with injection history (at a given dose).

Figure 3A shows rate of responding at the Polycose and sweetened ethanol levers, and Fig. 3B shows the proportion of responses at the lever that operated the sweetened ethanol dipper. In baseline and saline sessions, response rates maintained by ethanol and by Polycose were within one standard error on one another. Daidzin decreased both Polycose and sweetened ethanol reinforced responding [ $F(3,18) = 20.7, p < 0.001$ ]. The omnibus dose by type-of-drink interaction was not significant [ $p = 0.14$ ], but the

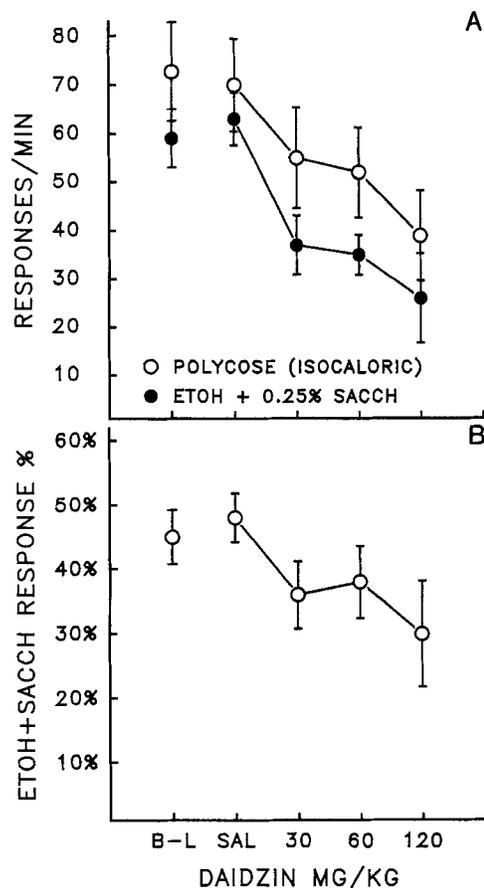


Fig. 3. On the x axis is dose and on the y axis is response rate (A) and the proportion of responses allocated to the ethanol lever (B). The results were calculated from the same sessions used to calculate the effects of daidzin on consumption. Error bars indicate standard errors of the mean.

quadratic dose by type-of-drink interaction was significant [ $F(1,6) = 6.4, p < 0.05$ ]. For example, the differences in response rates at the 30 and 60 mg/kg doses were larger than the difference at the 120 mg/kg dose.

Figure 3B shows the proportion of responses allocated to the lever that operated the ethanol dipper. There was a dose-dependent decrease in the proportion of responses at the ethanol lever [ $F(3,18) = 4.56, p < 0.02$ ], and post-hoc polynomial contrast tests showed that the decreases entailed a significant linear component [ $F(1,6) = 6.3, p < 0.05$ ], but no higher order components. Thus, the changes in choice proportions were somewhat more orderly than changes in absolute response rates (Fig. 3A data).

A blood sample was taken from each subject as described in "Methods." The blood ethanol readings ranged from 50 to 304 mg/dl, and the average value was 139 mg/dl.

## DISCUSSION

Daidzin decreased sweetened ethanol consumption more than it did starch consumption. Moreover, the decreases in ethanol consumption were large, reliable, and dose dependent. The pharmacological mechanisms that might have

mediated these effects have been the subject of several recent studies.<sup>5,7</sup>

Daidzin is a potent ( $K_i = 40$  nM) and selective inhibitor of human mitochondrial aldehyde dehydrogenase (ALDH),<sup>5</sup> an enzyme that catalyzes the detoxification of ethanol derived acetaldehyde.<sup>9</sup> Some humans inherit an inactive variant of this isozyme, and in these individuals alcohol abuse is rare.<sup>19</sup> These findings suggest that daidzin may influence alcohol consumption by mimicking the consequences of inactive ALDH. However, the exact mechanism is not known, and laboratory findings indicate that the relationship is complex. Sinclair and Lindros<sup>20</sup> found that calcium carbimide (an ALDH inhibitor) decreased ethanol consumption in rats, even though the rats had been treated with a drug (4-methylpyrazole) that prevented blood acetaldehyde accumulation. Similarly, daidzin, at doses that significantly suppressed ethanol consumption in Golden hamsters, did not affect overall acetaldehyde metabolism.<sup>7</sup> Based on these results, Keung and Vallee<sup>7</sup> suggested that a pathway other than that catalyzed by mitochondrial ALDH may be involved. However, they did not rule out the possibility that a physiological substrate of the mitochondrial ALDH, not acetaldehyde itself, may be involved in the regulation of drinking in these experiments.

In this context, it is of interest that Xie et al.<sup>21</sup> have shown that daidzin, whether fed to rats only once or chronically for 7 days, did not significantly alter either alcohol dehydrogenase or mitochondrial ALDH activity. These results suggest that the alcohol dehydrogenase-ALDH pathway does not mediate daidzin's antidipsotropic effects.

Recently Overstreet et al.<sup>22</sup> showed that a Chinese herbal medicine (NPI-028), which contains daidzin, also suppresses ethanol consumption in alcohol preferring (P) and Fawn-Hooded (FH) rats under a range of conditions. The effects of NPI-028 on acetaldehyde metabolism in these experiments was not studied. However, based on the observation that chronic NPI-028 administration did not induce a progressive reduction in alcohol intake in FH rats, the authors suggested that its alcohol intake-suppressing effect could not be mediated by the aversive effects of acetaldehyde. This conclusion is consistent with that of Keung and Vallee.<sup>7</sup>

Daidzin's effects on consumption may have been mediated by any of several behavioral mechanisms. Decreases in both ethanol and Polycose consumption suggest decreases in motor capacity and/or general appetite; larger decreases in ethanol consumption suggest changes in ethanol's oropharyngeal effects and/or more central actions. However, given that ethanol consumption differentially decreased, nonspecific changes in motor performance or appetite cannot be the whole story. Also, there were no obvious signs of motor deficits.

The blood-ethanol measures are similar to those obtained with this procedure in other studies.<sup>11</sup> However, there was no statistically significant correlation between ethanol consumption and blood ethanol level. This finding

was likely caused by the relatively narrow range of consumption levels. The rats consumed on average 2.45 g/kg of ethanol, but the variance in consumption was only 0.381 g/kg. In contrast, when ethanol consumption ranged widely, there was a strong linear relationship between ethanol intake and blood levels, using the same procedure as in this study.<sup>11</sup>

Finally, one detail of the results deserves some emphasis. The initial administration of daidzin produced the largest decrease in ethanol consumption, even though the dose was the lowest used, i.e., 30 mg/kg. Moreover, the 30 mg/kg dose had virtually no effect on Polycose consumption. Because there are no comparable studies, it is not clear whether this is a reliable effect. However, for all but one subject, the first 30 mg/kg dose was the most efficacious.

Daidzin has now been shown to decrease ethanol consumption in two species under quite different conditions. The hamsters had free access to chow (they were not food deprived), and they did not have to work (lever press) for ethanol. In contrast, the rats had to press a lever to get ethanol, they were food restricted, and they had access to a highly reinforcing and palatable starch solution. These comparisons suggest that daidzin's effects on ethanol consumption do not depend on either food restriction or diet. But perhaps the more important consideration is that daidzin is an ingredient of a long-used Chinese herbal treatment for alcohol abuse. This connection suggests that daidzin (or its congeners and/or metabolites) may also have an antialcohol effect in humans.

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