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Behavioural Pharmacological Characteristics of Honokiol, an Anxiolytic Agent Present in Extracts of Magnolia Bark, Evaluated by an Elevated Plus-maze Test in Mice

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Abstract

Honokiol, a neolignane derivative of Magnolia bark, has central depressant action and, at much lower doses, anxiolytic activity. We have investigated the characteristics of the behavioural effects of honokiol by means of an elevated plus-maze test.

In the plus-maze test a single oral dose of 20 mg kg^{-1} honokiol significantly prolonged the time spent in the open arms of the maze, suggesting anxiolytic effect. Moreover, when honokiol was administered daily for seven days and the plus-maze test was conducted 3 or 24 h after the last administration, significant prolongation of the time in the open arms was manifested even for doses of 0·2 mg kg^{-1}. The maximum effect was observed for doses of 0·5 mg kg^{-1}. Honokiol at any dose in both single and repeated administration schedules caused neither change in motor activity nor disruption of traction performance. Orally administered diazepam, 0·5–2 mg kg^{-1}, caused dose-dependent prolongation of the time spent in the open arms of the maze with a significant increase in motor activity at 1 mg kg^{-1}, and dose-dependent disruption of traction performance. The changes in the plus-maze performance after treatment for seven days with 0·2 mg kg^{-1} honokiol and after a single treatment with 1 mg kg^{-1} diazepam were almost equivalent. The effect of honokiol (0·2 mg kg^{-1}, treatment for seven days) was inhibited by subcutaneous flumazenil (0·3 mg kg^{-1}) and (+)-bicuculline (0·1 mg kg^{-1}) and by intraperitoneal CCK-4 (50 μg kg^{-1}) and caffeine (30 mg kg^{-1}). The anxiolytic effect of diazepam (1 mg kg^{-1}) was also inhibited by flumazenil and bicuculline. However, the combined administration of diazepam with caffeine enhanced the effect, and diazepam completely reversed the effect of CCK-4.

These results suggest that, in contrast with diazepam, honokiol selectively induces an anxiolytic effect with less liability of eliciting motor dysfunction and sedation or disinhibition. The combined effects of the drug also revealed that the mechanism of anxiolytic effect of honokiol is partially different from that of diazepam.

Honokiol is a neolignane derivative present in Magnolia bark (Fujita et al 1973). Watanabe et al (1975, 1983a, b) reported that honokiol had a central depressant action, successively higher doses eliciting muscle relaxation, sedation, sleeping and anaesthesia in mice. Maruyama et al (1998) have previously demonstrated by use of an improved apparatus for the plus-maze test (the floor of the open arms being transparent) (Kuribara & Maruyama 1996; Kuribara et al 1996) that at doses less than one hundredth of those eliciting central depressant action (Watanabe et al 1975, 1983a, b), treatment for seven days with honokiol extended the time spent in the open arms of the maze (suggesting anxiolytic action). However, further study is required to clarify the characteristics of the behavioural pharmacological effect of honokiol.

In this study we have conducted plus-maze, activity and traction tests to assess dose- and time-dependent changes in the effects of honokiol, and

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the combined effects of honokiol with drugs (diazepam, flumazenil and bicuculline) that have either agonistic or antagonistic action on the $GABA_A$-benzodiazepine receptor complex, or CCK-4 (de Montigny 1989; Bradwejn et al 1991, 1992a, b, 1994; Lydiard et al 1992; Bradwejn & Koszycki 1994; Crawley & Corwin 1994; Van Megen et al 1994; Price et al 1995) and caffeine (Uhde et al 1984; Charney et al 1985; Price et al 1995)) that are considered to induce panic attack-like symptoms. The effects of honokiol were compared with those of diazepam.

Materials and Methods

Drugs

Honokiol was obtained from Nacalai Tesque (Kyoto, Japan), diazepam and flumazenil from Hoffmann-La Roche (Nutley, NJ), (+)-bicuculline and anhydrous caffeine from Sigma (St Louis, MO) and cholecystokinin (30–33) ammonium salt (CCK-4) from ICN Biomedicals (Aurora, OH). The drugs were dissolved or suspended in physiological saline containing Tween-80 (1%). The concentration of each drug solution or suspension was adjusted so that the volumes administered were constant (0·01 mL g^{-1}).

Animals

Male mice of the BALB/c strain (Halan, IN), six-weeks-old, 22–27 g, were housed in groups of five in polycarbonate cages (width 15 cm, length 25 cm, height 12 cm) with wood-chip bedding; they were allowed free access to a solid diet and tap water. The conditions of the breeding room were controlled (temperature 23 ± 1°C; relative humidity 55 ± 3%; 12-h light–dark cycle, lights on between 0600 and 1800 h).

All experimental procedures were conducted according to the Guide for the Care and Use of Laboratory Animals and Animal Welfare Act, and the experimental protocol was approved by the Institutional Animal Care and Use Committee of the University of Texas Health Science Centre at San Antonio.

Apparatus and measurement procedures

The elevated plus-maze test. The elevated plus-maze used in this study was the same as that used in our previous studies (Kuribara & Maruyama 1996; Kuribara et al 1996) and was an improvement of the original apparatus for rats (Pellow et al 1985) and mice (Lister 1987). Briefly, the plus-maze consisted of two closed arms (width 6 cm, length 30 cm, height 10 cm) and two open arms (width 6 cm, length 30 cm) extending from a central platform (8 cm × 8 cm). The floor and side-walls of the closed arms, and the floor of the platform were made of non-transparent grey poly(vinyl chloride). In contrast, the open arms had no side-walls and the floor was made of transparent acrylic fibre. The plus-maze was set at a height of 40 cm.

Each mouse was placed on the central platform facing toward one of the closed arms. In the improved plus-maze apparatus, however, the mouse spent a significantly longer time in the closed arms, the time spent in the open arms and platform being very short. Therefore, the cumulative time spent in the open arms was recorded for 5 min, and this measurement was used as the index of plus-maze performance. The criterion for entry of the mouse into an open arm was crossing with all four paws of the borderline separating the open arm and the central platform.

Activity test. Immediately after the end of a plus-maze test the activity of the mouse was measured for 5 min with a tilting-type ambulometer with a bucket-like Plexiglas activity cage 20 cm in diameter (SMA-1; O'Hara, Tokyo). Each slight tilt of the activity cage caused by horizontal movement (positional change) of the mouse was detected by microswitches attached to the cage. Vertical movement of the mouse, such as rearing, head movement, sniffing, etc., and turning did not cause the activity cage to tilt.

Traction test. The traction test was conducted immediately after the activity test. The experimental procedure is described elsewhere (Kuribara et al 1977). Briefly, a wire (diameter 1·6 mm, length 30 cm) was set horizontally at a height of 30 cm. The mouse was first forced to grasp the wire with the four paws, and the duration of clinging to the wire was measured for up to 60 s. The trial was performed twice for each mouse, and the longer clinging time was used in the calculation of the mean value for each group of mice. When the duration of clinging was > 60 s the mouse was released from the wire and the clinging time was recorded as 60 s.

Administration schedules

Ten drug-naive mice were used in each experiment and all experimental treatment was conducted between 0900 and 1500 h.

Single administration. The behavioural tests were performed 1, 3 or 24 h after oral administration of honokiol (2 and 20 mg kg^{-1}) and 10 min after oral administration of diazepam (0·5, 1 and 2 mg kg^{-1}).

As the control experiment physiological saline containing Tween-80 was administered at the corresponding times.

Treatment with honokiol for seven days. The behavioural tests were conducted 3 h after oral administration of honokiol (0·2 mg kg^{-1}) or physiological saline containing Tween-80 for seven days or 24 h after similar treatment with honokiol (0·1, 0·2, 0·5, 1 and 2 mg kg^{-1}) or physiological saline containing Tween-80.

Combined administration of drugs. In experiments involving honokiol the drug (0·2 mg kg^{-1}) was administered orally, daily for seven days. Twenty-four hours after the last administration of honokiol the mice were given diazepam (1 mg kg^{-1}, p.o.), flumazenil (0·3 mg kg^{-1}, s.c.), bicuculline (0·1 mg kg^{-1}, s.c.), CCK-4 (50 μg kg^{-1}, i.p.) or caffeine (30 mg kg^{-1}, i.p.). Diazepam, flumazenil, bicuculline and CCK-4 were administered 10 min before the behavioural tests, caffeine 15 min before.

In experiments involving diazepam, diazepam (1 mg kg^{-1}, p.o.) was administered 10 min before the behavioural tests, and the challenge drugs were administered with the same schedules as in the honokiol study.

For comparison, four other groups of drug-naive mice were given either flumazenil (0·3 mg kg^{-1}, s.c.), bicuculline (0·1 mg kg^{-1}, s.c.), CCK-4 (50 μg kg^{-1}, i.p.) or caffeine (30 mg kg^{-1}, i.p.).

Statistical analysis

The time spent in the open arms in the plus-maze test, the activity counts in the activity test and the clinging time in the traction test were analysed by means of the Fisher PISD test. Values of $P < 0.05$ were regarded as indicative of significance.

Results

Single administration

Honokiol. As shown in Table 1, in the plus-maze test conducted 3 h after administration of 20 mg kg^{-1} honokiol significantly prolonged the time spent in the open arms. However, the effect had disappeared 24 h after administration. Honokiol at any dose did not change the motor activity or the traction performance.

Table 1. Effects of a single oral dose of honokiol on plus-maze, ambulatory activity and traction performance in mice.

Time after treatment	Dose	Plus-maze	Activity	Traction
		Time in the open arms (s)	Counts/5 min	Clinging time (s)
1 h				
Tween-80		13·2 ± 2·7	30·6 ± 1·7	60·0 ± 0·0
Honokiol	2 mg kg^{-1}	14·0 ± 6·0	28·1 ± 2·1	60·0 ± 0·0
	20 mg kg^{-1}	29·2 ± 10·4	30·8 ± 3·7	60·0 ± 0·0
3 h				
Tween-80		13·1 ± 4·7	31·2 ± 1·5	60·0 ± 0·0
Honokiol	2 mg kg^{-1}	21·7 ± 5·6	27·7 ± 3·9	60·0 ± 0·0
	20 mg kg^{-1}	38·2 ± 7·8*	25·9 ± 2·8	60·0 ± 0·0
24 h				
Tween-80		14·0 ± 2·6	26·2 ± 3·0	60·0 ± 0·0
Honokiol	2 mg kg^{-1}	16·0 ± 6·3	20·8 ± 1·2	60·0 ± 0·0
	20 mg kg^{-1}	11·6 ± 6·0	21·6 ± 1·9	60·0 ± 0·0

Values are means ± s.e., n = 10. *$P < 0.05$, significantly different from the result from the Tween-80-treated group.

Table 2. Effects of a single oral dose of diazepam on plus-maze, ambulatory activity and traction performance in mice.

Treatment	Dose	Plus-maze	Activity	Traction
		Time in the open arms (s)	Counts/5 min	Clinging time (s)
Tween-80		12·3 ± 2·6	24·9 ± 1·7	60·0 ± 0·0
Diazepam	0·5 mg kg^{-1}	29·7 ± 4·8*	28·2 ± 3·0	55·2 ± 4·8
	1·0 mg kg^{-1}	43·5 ± 6·1*	38·9 ± 3·2*	42·6 ± 6·3*
	2·0 mg kg^{-1}	137·7 ± 17·8*	30·6 ± 4·2	14·6 ± 2·0*

Values are means ± s.e., n = 10. Diazepam and Tween-80 were administered 10 min before the behavioural tests. *$P < 0.05$, significantly different from the result from the Tween-80-treated group.

Table 3. Effects of treatment with honokiol for seven days on plus-maze, ambulatory activity and traction performance in mice.

Time after treatment	Dose	Plus-maze	Activity	Traction
		Time in the open arms (s)	Counts/5 min	Clinging time (s)
3 h				
Tween-80		10·2 ± 3·7	30·9 ± 2·3	60·0 ± 0·0
Honokiol	0·2 mg kg^{-1}	49·8 ± 10·8*	31·8 ± 3·9	60·0 ± 0·0
24 h				
Tween-80		14·5 ± 5·2	30·3 ± 2·9	60·0 ± 0·0
Honokiol	0·1 mg kg^{-1}	26·8 ± 8·0	25·6 ± 3·8	60·0 ± 0·0
	0·2 mg kg^{-1}	43·0 ± 7·0*	27·1 ± 2·3	60·0 ± 0·0
	0·5 mg kg^{-1}	58·6 ± 13·2*	27·9 ± 3·8	60·0 ± 0·0
	1·0 mg kg^{-1}	41·2 ± 7·7*	29·9 ± 2·0	60·0 ± 0·0
	2·0 mg kg^{-1}	45·8 ± 6·8*	36·8 ± 2·6	60·0 ± 0·0

Values are means ± s.e., n = 10. *$P < 0·05$, significantly different from the result from the Tween-80-treated group.

Diazepam. As shown in Table 2, 0·5–2 mg kg^{-1} diazepam significantly and dose-dependently prolonged the time spent in the open arms. Diazepam at 1 mg kg^{-1} significantly increased activity and disrupted traction performance in a dose-dependent manner.

Treatment with honokiol for seven days

Table 3 shows the results obtained after treatment with honokiol for seven days and from behavioural tests performed 3 or 24 h after the last administration. Although the single dose of 0·2 mg kg^{-1} honokiol did not change the plus-maze performance, treatment for seven days resulted in significant extension of the time spent in the open arms when the test was performed 3 h after the last administration. When the elevated plus-maze test was conducted 24 h after the last administration, doses of honokiol up to 0·5 mg kg^{-1} dose-dependently prolonged the time spent in the open arms.

Table 4. Effects of oral honokiol (0·2 mg kg^{-1}, seven-day treatment) or diazepam (1 mg kg^{-1}), either alone or in combination with other drugs, on plus-maze, ambulatory activity and traction performance in mice.

Treatment	Plus-maze	Activity	Traction
	Time in the open arms (s)	Counts/5 min	Clinging time (s)
Tween-80	12·3 ± 2·6	24·9 ± 1·7	60·0 ± 0·0
Honokiol (0·2 mg kg^{-1})	43·0 ± 7·0*	27·1 ± 2·3	60·0 ± 0·0
Diazepam (1·0 mg kg^{-1})	43·5 ± 6·1*	38·9 ± 3·2*	42·6 ± 6·3*
Honokiol + diazepam	102·3 ± 13·6†	29·8 ± 4·4†	43·8 ± 4·9*
Flumazenil (0·3 mg kg^{-1})	6·7 ± 2·9	25·6 ± 3·0	60·0 ± 0·0
Honokiol + flumazenil	9·7 ± 5·1†	25·5 ± 2·3	60·0 ± 0·0
Diazepam + flumazenil	13·5 ± 3·8†	27·9 ± 1·8†	54·5 ± 2·9*†
Bicuculline (0·1 mg kg^{-1})	7·1 ± 2·8	21·5 ± 2·7*	60·0 ± 0·0
Honokiol + bicuculline	10·7 ± 4·7†	20·9 ± 1·4*†	60·0 ± 0·0
Diazepam + bicuculline	17·4 ± 4·9†	37·0 ± 2·3*	46·6 ± 6·9*
CCK-4 (50 μg kg^{-1})	2·2 ± 1·1*	29·2 ± 2·2	60·0 ± 0·0
Honokiol + CCK-4	9·3 ± 3·2†	33·8 ± 3·0	60·0 ± 0·0
Diazepam + CCK-4	57·3 ± 13·0*	34·1 ± 5·1	41·3 ± 7·7*
Caffeine (30 mg kg^{-1})	13·9 ± 9·0	60·5 ± 7·4*	60·0 ± 0·0
Honokiol + caffeine	31·0 ± 8·0*	63·7 ± 4·4*†	60·0 ± 0·0
Diazepam + caffeine	93·8 ± 11·3*†	55·1 ± 7·1*†	50·4 ± 5·1

In the honokiol study, 24 h after the last administration of honokiol, bicuculline, CCK-4 or caffeine was administered and the behavioural tests were conducted 10, 10, 10 or 15 min, respectively, after administration. In the diazepam study, diazepam, bicuculline and CCK-4 were administered 10 min before, and caffeine 15 min before the behavioural tests. Values are means ± s.e., n = 10. *$P < 0·05$, significantly different from the result from the Tween-80-treated group. †$P < 0·05$, significantly different from the result from the honokiol- or diazepam-treated groups.

When the dose of honokiol was 1 or 2 mg kg^{-1}, the potency was slightly lower than when the dose was 0·5 mg kg^{-1}. Neither motor activity nor traction performance was affected by any treatment with honokiol.

Combined drug administration

Table 4 shows the results obtained after combined administration of honokiol or diazepam with various drugs. Because the results obtained after treatment with Tween-80 10 and 15 min before the behavioural tests were almost identical, the data obtained after administration of Tween-80 10 min before testing are presented in Table 4.

Combined treatment with honokiol and diazepam significantly enhanced the effect on the plus-maze performance. In contrast, the diazepam-induced increase in motor activity, but not disruption of traction performance, was reduced by honokiol.

Flumazenil at 0·3 mg kg^{-1} reduced the time spent in the open arms in the plus-maze test, without affecting motor activity or traction performance. Honokiol- and diazepam-induced prolongation of the time spent in the open arms was inhibited by flumazenil. The diazepam-induced increase in motor activity and disruption of traction performance were also ameliorated by flumazenil.

Bicuculline at 0·1 mg kg^{-1} had no effect on the plus-maze test, but significantly reduced motor activity. The effect of honokiol and diazepam on the plus-maze performance was inhibited by bicuculline. Motor activity after treatment with honokiol plus bicuculline was almost the same as that after bicuculline treatment alone. Bicuculline did not ameliorate either the diazepam-induced increase in the motor activity or disruption of traction performance.

CCK-4 at 50 μg kg^{-1} reduced the time spent in the open arms without marked change in motor activity or traction performance. In the plus-maze test combined treatment with honokiol and CCK-4 resulted in an antagonistic interaction. Thus, the mean time spent in the open arms was found to be shorter than the time spent after a single treatment with honokiol but longer than that spent after a single treatment with CCK-4 alone. However, diazepam completely reversed the effect of CCK-4. The diazepam-induced increase in motor activity and the disruption of traction performance were not significantly affected by CCK-4.

Caffeine at 30 mg kg^{-1} had no effect on plus-maze performance but significantly increased motor activity. Although the honokiol-induced prolongation in the time spent in the open arms tended to be reduced by caffeine, the time spent in the open arms after combined treatment with diazepam and caffeine was significantly longer than after treatment with diazepam alone. Motor activity after treatment with honokiol or diazepam plus caffeine was almost the same as that after treatment with caffeine alone. Caffeine tended to ameliorate the diazepam-induced disruption of traction performance.

Discussion

The validity of elevated plus-maze for testing anxiolytic drugs is well established (Dawson & Tricklebank 1995; Kulkarni & Reddy 1996). In the test the times spent in closed arms, open arms and platform, and the number of entrances to closed and open arms have generally been used as indices of the anxiety. In this study, using the improved plus-maze with a transparent floor in the open arms, only the time spent in the open arms was used, because the time the mouse spent in the open arms and platform was significantly shorter than that spent in the closed arms.

This study first confirmed our preliminary findings, based on the plus-maze test, that treatment with honokiol prolongs the time spent in the open arms, i.e. led to anxiolytic activity (Maruyama et al 1998), despite the use of different strains of mice. A single treatment with honokiol elicited significant anxiolytic effect at 20 mg kg^{-1}, less than the minimum effective dose for central depressant action, for example muscle relaxation and sedation (Watanabe et al 1975, 1983a, b). It is, therefore, very interesting to note that treatment with 0·2 mg kg^{-1} honokiol for seven days resulted in significant anxiolytic effect 3 and 24 h after the last administration. Thus, the anxiolytic effect after treatment with honokiol for seven days was more than 100 times greater than that after a single administration. Maruyama et al (1998) have reported that daily treatment with honokiol (0·2 mg kg^{-1}) for five or more days was required for development of the anxiolytic effect. However, it has been reported that the half-lives of honokiol after intravenous administration to rats were 49·22 min (5 mg kg^{-1}) and 56·24 min (10 mg kg^{-1}) (Tsai et al 1994). Moreover, the anxiolytic effect after treatment with honokiol for seven days was maximum for a dose of 0·5 mg kg^{-1}. These results indicate that, rather than a direct effect of honokiol, an increase in the metabolites of honokiol or changes in neuronal function is involved in the honokiol-induced anxiolytic effect. Hattori et al (1984a, b) reported that daily treatment with magnolol, an isomer of honokiol derived from Magnolia bark, resulted in a

progressive increase in the plasma concentration of hydrogenated magnolol in rats. Our preliminary study showed that honokiol did not inhibit [^{3}H]muscimol-binding to $GABA_A$ receptors (unpublished data).

Consistent with many reports (Pellow & File 1986; Kulkarni & Shaema 1991; Dawson & Tricklebank 1995; Kulkarni & Reddy 1996), diazepam resulted in a dose-dependent anxiolytic effect. Comparison of the dose–effect relationship after treatment with honokiol for seven days (with behavioural observation being performed 24 h after the last administration) or a single treatment with diazepam revealed that the anxiolytic activity of 0·2 mg kg^{-1} honokiol was almost equivalent to that of 1 mg kg^{-1} diazepam. Therefore, when the drugs were administered in combination, modification by the challenge drugs of the effects of such drug treatments with honokiol and diazepam were evaluated.

Flumazenil shortened the time spent in the open arms in the plus-maze test, showing an anxiogenic effect. The anxiogenic effect of the benzodiazepine receptor antagonist flumazenil has been reported in animal studies (Haefely 1988; Lee & Rodgers 1991) and in clinical trials (Nutt et al 1990; Woods et al 1991; Kapczinski et al 1995; Price et al 1995). Dalvi & Rodgers (1996) reported that low doses of the $GABA_A$ receptor antagonist bicuculline had no effect on the plus-maze in mice. Our results from determination of the effects of 0·3 mg kg^{-1} flumazenil and 0·1 mg kg^{-1} bicuculline on mice in the plus-maze test were consistent with these reports. As demonstrated by the antagonistic interaction of benzodiazepines and bicuculline (Boast et al 1983; Quintero et al 1985), the anxiolytic effect of diazepam was inhibited not only by flumazenil but also by bicuculline. The diazepam-induced increase in motor activity and disruption of traction performance were also ameliorated by flumazenil. Both flumazenil and bicuculline strongly inhibited the anxiolytic effect of honokiol. Furthermore, significant enhancement of the anxiolytic effect was elicited by combined treatment with honokiol and diazepam. These results clearly indicate that the $GABA_A$-benzodiazepine receptor complex is involved in the anxiolytic effect of honokiol after repeated administration.

However, combined administration with either CCK-4 or caffeine revealed different behavioural characteristics of honokiol and diazepam. Consistent with the clinical evidence as the panicogenic agent (de Montigny 1989; Bradwejn et al 1991, 1992a, b, 1994; Lydiard et al 1992; Bradwejn & Koszycki 1994; Price et al 1995) and anxiogenic agent in animal models (Harro et al 1990; Harro & Vasar 1991; Singh et al 1991), CCK-4 shortened the time spent in the open arms in the plus-maze test. The anxiogenic effect of CCK-4 was completely reversed by diazepam. This result is consistent with the clinical practice of treating panic disorder with benzodiazepines (Shader & Greenblatt 1995). In contrast, the anxiogenic effect of CCK-4 was only partially reduced by honokiol, suggesting that honokiol is less effective in the treatment of panic disorder than are benzodiazepines. It has also been reported that caffeine can increase the likelihood of panic attack in patients suffering from panic disorder (Uhde et al 1984; Charney et al 1985), and induces anxiety-like behaviour in animals (Baldwin & File 1989; Jain et al 1995). Although the plus-maze test did not reveal the anxiogenic effect of caffeine, compared with the effect of honokiol alone the combination of honokiol and caffeine reduced the anxiolytic effect of honokiol. In contrast, combined administration of diazepam and caffeine enhanced the anxiolytic effect. Similar enhancement of anxiolytic effect has been reported after combined administration of chlordiazepoxide and caffeine before the conflict test (Coffin & Spealman 1985), although benzodiazepines and methylxanthines sometimes interact antagonistically according to many behavioural indicators (Phillis & Wu 1982). The interaction of honokiol and caffeine demonstrated in this study also suggests that honokiol might not be appropriate for treatment of panic disorder.

Honokiol never affected motor activity and did not disrupt the traction performance at any dose either in the single treatment schedule or after treatment for seven days. However, diazepam increased motor activity and disrupted the traction performance. These effects of diazepam might be correlated with disinhibition and muscle relaxation and with ataxia and motor impairment, respectively. Furthermore, at doses greater than 5 mg kg^{-1}, diazepam induced sedation (data not presented). In this respect, honokiol is likely to have selective and potent anxiolytic effect without eliciting behavioural disorders such as sedation/disinhibition, muscle relaxation, ataxia and motor impairment, which are generally induced as side effects of benzodiazepine (Schweizer et al 1995; Woods & Winger 1995; Woods et al 1995). Two distinct mechanisms are possible—honokiol or its metabolites selectively stimulate the $GABA_A$-benzodiazepine receptor subtypes which are responsible for the anxiolytic/anxiogenic effect or honokiol binds to other sites related to the anxiolytic effect. Thus, further studies, including neurochemical and pharmacokinetic evaluation, are

required for precise elucidation of the mechanisms of the action of honokiol.

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