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Psychoneuroimmunological Effects of Dioscorea in the Ovariectomized Rats: Role of Anxiety Level

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Abstract

The anxiety level in the rats is correlated to the interleukin-2 (IL-2) in the brain. The present study aimed at investigating the effects of dioscorea (will yam), a Chinese medicine, on the emotional behavior and the IL-2 level in the brain of ovariectomized (OVX) rats. One month after the ovariectomy, female Wistar rats were screened by using the elevated plus-maze (EPM) test for measuring the anxiety level and were then orally administered by dioscorea (250, 750, and 1500 mg/kg/day). Three weeks later, these animals were then tested again in the EPM and in the forced swim (FS) test. The anxiety level was highly increased in a half of the OVX rats, but not in the other half one. Chronic administration of dioscorea, at doses of 750 and 1500 mg/kg/day, exerted anxiolytic activity in HA OVX rats. However, lower dose of dioscorea, 250 mg/kg/day, increased the anxiety level in LA OVX rats. These behavioral data were compatible with the findings in IL-2, where the IL-2 level in the cerebral cortex of HA OVX rats was significantly attenuated by the treatment of dioscorea, but the IL-2 level in the prefrontal cortex of LA OVX rats was enhanced by the dioscorea at 250 mg/kg/day. Learned helplessness in the FS test was inhibited by the dioscorea at 1500 mg/kg/day. The present results provide new insight into the role of IL-2 in the individual differences of anxiety in the postmenopausal animals. The psychoneuroimmunological function needs to be taken into account when measure the behavioral effects of dioscorea.

Keywords: dioscorea, anxiety, depression, individual differences, elevated plus-maze, cytokine, interleukin, ovariectomy, menopause

Background

Anxiety and depression are very common in postmenopausal women. Decreasing level of blood sexual hormone are proposed to be involved in these disorders(Davidson, 1985) because the postmenopausal syndrome is significantly improved by hormone replacement therapy(Linzmayer et al., 2001). Interleukin-2 (IL-2) has recently been implicated as a modulator of brain neuronal function and in the emotional behavior(Petitto et al., 1997; Hanisch, 2001), when cytokine functions in the brain and periphery was manipulated pharmacologically(Zalcman et al., 1998; Koh and Lee, 2004). Anxiogenic activity caused by the systemic administration of IL-2 also supports this view(Koh and Lee, 2004).

Sexual hormones, for example estrogen and progesterone, are able to modify emotional behavior of ovariectomized (OVX) Long-Evans rats, decreasing anxiety, fear, and pain responses, through actions in certain brain areas(Frye and Walf, 2004). In addition, estrogen and / or progesterone administered systemically have anxiety-, fear-, and pain-reducing effects in OVX rats(Frye and Walf, 2004). Dioscorea has long been used as a Chinese medicine for improving gastrointestinal, sensory, memory, sexual-related functions, and also the hot flush and frequency of urination in postmenopausal women. Basic researches have evaluated the effects of dioscorea on the osteoporosis(Yin et al., 2003), diabetes(Iwu et al., 1990), and hyperlipidemia(Chen et al., 2003), which are also very common in postmenopausal women; but no research investigating the role of dioscorea in the psychoneuroimmunological function has been published. Diosgenin, the predominant steroidal saponin in dioscorea(Marker et al., 1940; Marker et al., 1943), is used to manufacture steroidal hormones such as progesterone, estrogen, testosterone, and cortisone(Marker, 1940b; Rosenkranz et al., 1951) by in vitro chemical modification(Marker, 1940a); however, the recent studies perform on menopausal animals indicate that the levels of sexual hormone may not be affected by the treatment of diosgenin(Benghuzzi et al., 2003). Further, the diet supplement of dioscorea did not influence the dehydroepiandrosterone level in the blood(Araghiniknam et al., 1996). Therefore, it was hypothesized that, in addition to being a precursor of sexual hormone in vivo, other mechanisms, for example psychoneuroimmunological function, may also be involved in the effects of dioscorea on the menopausal symptoms.

Previous study indicates that there is anti-inflammatory action of dioscorea in vivo(Lee et al., 2002) and in vitro, decreasing the production of cytokines(Kim et al., 2004). Little is known, however, about the role of IL-2 in the menopausal syndrome. Since the IL-2 in the brain participates in the emotional behavior, and is area dependent(Petitto et al., 1997;

Connor et al., 1998), it deserve to investigate the effects of dioscorea on the level of IL-2 in the brain of menopausal animals.

The OVX rats are used as the menopausal animal model because the changes of biochemical and physiological function are comparable with that in menopausal women(Bosse and Di Paolo, 1995), that is, decreasing the levels of progesterone and estrogen(Erb et al., 1968), increasing the risk of cardiovascular disease(Sharkey et al., 1999), and enhancing the rate of bone loss(Higdon et al., 2001; Katase et al., 2001), as well as increasing anxiety level(Fernandez-Guasti et al., 2001). In addition, the time spent in the open arm during the elevated plus-maze (EPM) test is used to evaluate unconditioned avoidance behavior as a measure of anxiety (Pellow et al., 1985; Blanchard et al., 1990); furthermore, the immobility, a despair behavior, in the forced swim (FS) test, is used to investigate learned helplessness as an animal model of depression (Porsolt et al., 1977). To our knowledge, work on the role of dioscorea for individual differences in anxiety and depression caused by OVX has not yet been published. With the goal of understanding whether the responses of emotional behavior and IL-2 function in OVX rats to dioscorea treatment may differ between low (LA) and high (HA) anxiety rats, we screened a group of OVX rats by using the EPM test and then measured their behavioral responses in the EPM as well as FS test after chronic treatment of dioscorea. To elucidate the effects of dioscorea on the level of IL-2 in the brain tissue, an enzyme-linked immunoassay (ELISA) was performed.

Materials and Methods

Animals

Female Wistar rats (261 ± 4 g; n=99; National Laboratory Animal Center, ROC) were used and housed in groups of five rats in acrylic cages (35 × 56 × 19 cm) in an animal room with a 12 hr light-dark cycle (lights on at 07:00 hr) with food and water provided *ad libitum*. Each animal was handled, 15 min/day, on 2 consecutive days prior to the experiment. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of the Chung Shan Medical University.

General procedure

Four weeks after the ovariectomy, open field test (10 min) and then EPM test (5 min) were performed on two consecutive days. The animals were, then, orally administered by dioscorea (0, 250, 750, or 1500 mg/kg/day) for 27 days. Three weeks after the treatment of dioscorea, the EPM test was again performed and then a 2-day session of FS test were taken (see detail in Table 1). All behavioral tests were begun 2 hr after the start of the lights on and were performed before the dioscorea treatment of that day. First, the animals were weighed in the animal room, placed individually in a clean cage (25 × 41 × 19 cm), and transported to a dim observation room (28 lux) for behavioral testing. The pieces of test equipment were thoroughly cleaned by using 20% alcohol followed by thorough drying before each rat was tested. The behavioral parameters of the open field test were analyzed by an automated computer program; behavior in the EPM and the FS test was scored from videotapes.

Ovariectomy

Aseptic surgical technique was employed for all animals. Rats were anesthetized by using ketamine (100 mg/kg, IM). The dorsal part of the lumbar was shaved, and the site was cleaned with 75% ethanol followed by a thorough scrubbing with 10% povidone iodine. A 2 cm incision was made into the skin, through the musculature and peritoneum. The ovaries were retracted and removed. The wound was then closed using 4-O sterile suture. Immediately following the surgery, each rat was injected with Penicillin-G procaine (0.2 ml, 20,000 IU, IM), and the wounds were once again cleaned with povidone iodine to reduce the chance of post-operative infection. The sham-operated group underwent the same surgical procedure except for the removal of the ovaries. After OVX, the rats were kept individually in plastic cages (25 × 41 × 19 cm) for recovery for about 10 days,

Behavioral tests

Open field: The open field consists of an acrylic box (40 × 40 × 40 cm). The movement distance of rats in a 10 min observation was monitored by an automated activity monitoring system (Digiscan-16 Animal Activity Monitor System; model RXYZCM, Omnitech Electronics Columbus, OH, USA) (Ho et al., 2000).

EPM test: The EPM apparatus was made of plastic and consisted of two opposed open arms (50 × 10 cm), two opposed enclosed arms with no roof (50 × 10 × 40 cm), and an open square (10 × 10 cm) in the center; it was elevated 50 cm above the floor. Behavior in the

EPM was observed for 5 min as described before (Ho et al., 2002). The following measures were analyzed from videotapes: (1) arm time: the time spent on open or enclosed arms, (2) arm entries: the number of entries into open or enclosed arm, and (3) arm activity: the number that an animal crossed a virtual line which divided an arm into a proximal and a distal half. An entry into any of the compartments was defined as all four paws being placed on the compartment. The EPM test was performed twice in this study: one was taken 4 weeks after ovariectomy; the other was taken 3 weeks after the dioscorea treatment. The open arm time in the first EPM test was used to screen individual anxiety levels and to arrange treatment groups which were matched for equivalent numbers of high and low open arm responders.

FS test: This test was carried out in a clear glass tank (25 × 25 × 60 cm) containing 39 cm clean water (26 ºC). The apparatus was cleaned thoroughly, and water was changed from rat to rat. A 2-day swimming session was conducted and videotaped: 15 min on the first day and 5 min on the second day as described before(Ho et al., 2005). The immobility were measured from videotapes, which occurred when the rats remained motionless, or floating (including small limb movements to keep their heads above the water) (Armario et al., 1991). For elucidating the role of FS test on the IL-2 level, one third of rats did not receive the FS test, based on random assignment.

Measurement of IL-2

Two days after the FS test, the rats were sacrificed by exposure to CO_2 vapors and their brains were removed immediately; the prefrontal cortex and cerebral cortex were dissected out on an ice-bath plate. The protein in the tissue was extracted by homogenizing the collected tissues in ice-cold lysis buffer (50 mM Tris-HCl, pH7.6, 0.5% NP-40, 150 mM NaCl, 1 mM EDTA, 10% glycerol containing protease inhibitors (1 μg/ml of aprotinin, 0.5 μg/ml of leupeptin, and 100 μg/ml of 4-(2-aminoethyl) benzenesulfonyl fluoride)). After centrifugation at (13,000 rpm × 15 min, twice, 4℃) (Hermle Z323K centrifuge, Gosheimerstr, Germany), the protein in the supernatant was harvested; its' concentration was determined using a Bio-Rad protein assay kit (Bio-Rad laboratories, CA, USA). A total of about 30-40 μg protein was subjected to detect the IL-2 level by using an ELISA kits, with monoclonal anti-mouse IL-2, according to the manufacturer's instructions. The color reaction was stopped by (TMS One-Step Substrate reagent) H_2SO_4 at 30 min, and the optical density was read at 450 nm within 30 min by an ELISA reader. The IL-2 level was then calculated from the standard curves. Sensitivity of this ELISA is 1 pg/mL, intraassay variability ranges from 4% to 11%, and interassay variability is 7.0%.

Dioscorea:

Dioscorea (D. L. alata. Var. purpurea (Roxb.) M. Pouch; Tainung No. 1 Shan-Yao) was purchased from Ming-Jean village, Nan Tao County, Taiwan. The yam tubers were cleaned, peeled to remove the outside skin, sliced to 1 cm width slices, then cooked for 30 min to inhibit the browning reaction. Put the cooked sample, thereafter, to the moisture to around 10%, milled to the flour that pass through 60 mesh sieve, and then stored at -25℃ until the use. The dioscorea dosage was freshly prepared before the use by adding and mixing with double distilled water.

Data analysis: Identically to our previous experiments(Ho et al., 2002), the OVX rats were ranked using the open arm time in the first EPM test and were assigned to two subgroups with either high (the 34 animals with shorter open arm time; HA rats) or low anxiety levels (the other 34 animals with longer open arm time; LA rats). This group assignment was used to present the dioscorea effects on behavior and IL-2. Statistical testing was performed to compare within or between groups using *t*-tests for paired or unpaired data. The comparison of dioscorea effects was carried out by one-way analysis of variance (ANOVA), followed by least-significant (LSD) test. All results were expressed as the mean ± SEM. The level of significance was defined as $p < 0.05$.

Results

Behavior after ovariectomy

Four weeks after ovariectomy, the open arm time in OVX rats (30.8 ± 4.0 sec) was shorter than in sham-operated rats (50.6 ± 7.4 sec) (df = 97, $t = 2.576$, $p = 0.012$). But the enclosed arm time was longer in OVX rats (241.1 ± 5.1 sec) than in sham-operated rats (215.7 ± 9.5 sec) (df = 97, $t = 2.576$, $p = 0.012$). The total arm activity in the EPM test (27.2 ± 2.1 vs. 28.4 ± 1.2) and the movement distance in the open field test (2644 ± 129 cm vs. 2903 ± 132 cm) were not different between OVX and sham-operated rats.

The OVX rats were divided into HA and LA subgroups, based on the open arm time in the first EPM test(Ho et al., 2002). These subgroups had the following profiles: The

behavior in the open arm, which was presented as the open arm time, and the open arm activity, was significantly lower in HA rats than in LA rats (all *p*-values < 0.001). But the HA rats showed more behaviors in the enclosed arm, which was expressed as higher enclosed arm time and enclosed arm activity, compared to LA rats (all *p*-values < 0.05). Total arm activity, however, was not different between HA and LA rats. Interestingly, all the above parameters in LA rats were the same with that in sham-operated rats (Table 2).

Behavior after dioscorea treatment

Dioscorea did not influence the behavior of sham-operated rats in the EPM test (Table 3), but significantly changed the EPM behavior of OVX rats. Chronic treatment with dioscorea, 750 mg/kg/day, for 3 weeks, significantly influenced the EPM behavior in OVX HA rats, increasing the open arm time and open arm activity (*p*-values < 0.001) but decreasing the enclosed arm time and enclosed arm activity (*p*-values < 0.05), compared with the data before the treatment. Similar effects were also observed at dosage of 1500 mg/kg/day, elevating the open arm time but suppressing the enclosed arm time (*p*-values < 0.05). Interestingly, however, dioscorea at 250 mg/kg/day significantly decreased the open arm behavior of OVX LA rats, expressed as the attenuations of open arm time and open arm activity (*p*-values < 0.05). All the dosage of dioscorea used in this study didn't affect the total arm activity in EPM test (Table 4).

***FS test*:**

The immobility time during the 1st 5-min period of FS test in the day-1 session in the OVX rats treated with vehicle and 750 mg/kg dioscorea was significantly higher than that in sham-operated rats treated with identical dosage (*p*-values < 0.01, *t*-test). The learned helplessness was observed in all the rats, the immobility time in day-2 session being significantly longer than that in day-1 session (*p*-values < 0.05), except that receiving 1500 mg/kg/day of dioscorea in the OVX group (Figure 1).

***IL-2 measurement*:**

The IL-2 data from all rats were taken into account, irrespective of receiving FS test or not, because the IL-2 level was not different between these tow groups. Dioscorea didn't affect the IL-2 level in the prefrontal cortex and cerebral cortex in sham-operated rats. The IL-2 level in the prefrontal cortex of vehicle-treated OVX LA rats was lower than that in the vehicle-treated sham-operated rats ($p < 0.05$); however, it was reversed by the treatment of

dioscorea at the dosage of 250 mg/kg/day ($p < 0.05$). In addition, all the 3 dosages used in this study, 250, 750, and 1500 mg/kg/day, significantly decreased the IL-2 level in the cerebral cortex of OVX HA rats, compared with vehicle-treated OVX HA rats (p-values < 0.05) (Table 5).

***Analysis of animals with low (LD) vs. high distance (HD) levels in the open field*:** In addition to the analysis based on the open arm time in the EPM test, all animals were divided into LD and HD rats based on the movement distance in the open field test. When analyzing the behavior in EPM and FS test, there were no indications for substantial differences between LD and HD rats; and also, the effects of dioscorea in LD and HD groups were not different (data not shown in detail).

Discussion

The present experiment showed that one month after ovariectomy anxiety level was highly increased in a half of the rats, but not in the other half one. Chronic administration of dioscorea, at doses of 750 and 1500 mg/kg/day, exerted anxiolytic activity in HA OVX rats, increasing the open arm time and decreasing the enclosed arm time in the EPM test. However, lower dose of dioscorea, 250 mg/kg/day, increased the anxiety level in LA OVX rats. These behavioral data were compatible with the findings in IL-2, where the IL-2 level in the cerebral cortex of HA OVX rats was significantly attenuated by the treatment of dioscorea, but the IL-2 level in the prefrontal cortex of LA OVX rats was enhanced by the dioscorea at 250 mg/kg/day. These effects of dioscorea were not due to unspecific effects on activity, since the dosages used did not affect total arm activity in the EPM test. In addition, learned helplessness in the FS test was inhibited by the dioscorea at 1500 mg/kg/day. The present data suggest that IL-2 in the brain plays a pathophysiological role in the postmenopausal anxiety and is involved in the mechanisms by which dioscorea influences the emotional behavior in the OVX rats.

The OVX rats are used as the menopausal animal model since the changes of biochemical and physiological function are comparable with that seen in menopausal women(Bosse and Di Paolo, 1995), that is, decreasing the levels of progesterone and estrogen(Erb et al., 1968), increasing the risk of cardiovascular disease(Sharkey et al., 1999), and enhancing the rate of bone loss(Higdon et al., 2001; Katase et al., 2001). Although the

anxiety and depression were thought to be very common in menopausal women and that the elevation of anxiety level was also reported in Long-Evans(Zimmerberg and Farley, 1993) and Wistar rats(Fernandez-Guasti et al., 2001) after ovariectomy, the present study indicates that only half of OVX rats was denoted as high anxiety. This finding is coincident with observations in clinical research, the anxiety being not necessary appeared in postmenopausal women(Sagsoz et al., 2001).

As our knowledge, the present data is the first one reporting that dioscorea has the effects on the psychoneuroimmunological function. Dioscorea has long been used as a Chinese medicine for improving gastrointestinal, sensory, memory, and sexual-related functions. Several lines of evidence have demonstrated that dioscorea is effective in the treatment of osteoporosis(Yin et al., 2003), diabetes(Iwu et al., 1990), and hyperlipidemia(Chen et al., 2003); but lack of research investigating the role of dioscorea in psychoneuroimmunological function has been published. Our data show that oral administration of dioscorea to the menopausal animals not only influenced the emotional behavior but also reversed the IL-2 level in the brain.

Sexual hormone system may participate in the behavioral effects of dioscorea. Decreasing level of blood sexual hormone are proposed to be involved in disorders after menopause(Davidson, 1985) because the postmenopausal syndrome is significantly improved by hormone replacement therapy, especially by the combined estrogen-progesterone regimen(Linzmayer et al., 2001). Diosgenin, the predominant steroidal saponin in dioscorea(Marker et al., 1940; Marker et al., 1943), is used to manufacture steroidal hormones such as progesterone, estrogen, testosterone, and cortisone(Marker, 1940b; Rosenkranz et al., 1951). Although the literature is lacking in the exact mechanisms by which the diosgenin converses to another hormones in vivo, previous researches indicate that the hypertrophy of the adrenal cortex in OVX animals was reversed back toward control values after continuous supplementation with diosgenin(Benghuzzi et al., 2003). Further, the consumption of wild Mexican yam products containing diosgenin increased the progesterone activity of saliva(Zava et al., 1998), suggesting that the functions of steroidal hormone were influenced.

IL-2 has recently been implicated as a modulator of neuronal function and in the emotional behavior(Petitto et al., 1997). Pharmacological manipulations of cytokine function in the brain and periphery have been shown to affect behavior(Zalcman et al., 1998; Koh and Lee, 2004). Evidence shows that the IL-2 can influence emotional behaviors(Hanisch, 2001); the anxiogenic activity was observed after the systemic administration of IL-2(Koh and Lee, 2004). In addition, IL-2/15Rβ knockout mice

compared to wild-type and heterozygote mice exhibited decreased levels of anxiety-like behaviour in the EPM test(Petitto et al., 2002). Chronic administration of IL-2 produced reductions in exploration and approach to a novel stimulus, indicating the correlation between IL-2 and the defensive behavior(Lacosta et al., 1999). In the present study, the decreased avoidance to the open arm of EPM was compatible with the lowered level of IL-2 in the cerebral cortex of HA OVX rats treated by dioscorea. In contrast, the increased defensive behavior was relevant to the augmentation of IL-2 level in the prefrontal cortex of LA OVX rats after the treatment of dioscorea at 250 mg/kg/day.

The current data support the view that the function of cytokines is area-specific(Petitto et al., 1997). Previous study indicated that intracerebroventricular administration of IL-1beta and tumor necrosis factor-alpha provoked an anxiogenic response in the EPM test without affecting the neurotransmitter concentrations in the amygdala(Connor et al., 1998). However, the dopaminergic system in the striatum is reported to be sensitive to the modulation of IL-2(Petitto et al., 1997). The present study showed that dioscorea elevated the anxiety and IL-2 in the prefrontal cortex of LA OVX rats but attenuated the anxiety and IL-2 in the cerebral cortex of HA OVX rats. Because several areas of the brain take part in the modulation of emotional behavior, the function of IL-2 in these areas is worthy to study further.

The immobility time in the day-1 session of FS test was higher in OVX rats than in sham-operated one, suggesting that the basal level of despaired behavior is higher in OVX rats. In addition, the learned helplessness was blocked by dioscorea at only highest dosage, but the anxiolytic effects were observed at all the doses used, showing that the biological basis of anxiety and depression is not identical(Ho et al., 2002; Ho et al., 2005). Further, the effects of dioscorea on the behavior and IL-2 depend on the anxiety level of OVX rats, implying that the function of cytokines may be involved in the individual differences of anxiety. In addition, the behavioral effects of dioscorea depended on the anxiety level because the anxiety behavior in the EPM test was increased in LA rats but decreased in HA rats. This view is also supported by the finding that when divided animals into HD and LD sub-groups based on their movement distance in the open field, the dioscorea effects were not different between HD and LD rats.

Conclusion

Anxiety level was highly increased in a half of OVX rats, but not in the other half one. The anxiolytic activity of chronic treatment of dioscorea was correlated to the decreasing of IL-2 level in the cerebral cortex of HA OVX rats. Contrarily, the anxiogenic effects of

dioscorea in the LA OVX rats were accompanied with elevation of IL-2 level in the prefrontal cortex. In addition, the learned helplessness in the FS test was blocked by the dioscorea at only highest dose. The present results provide new insight into the role of IL-2 in the individual differences of anxiety in the postmenopausal animals. The psychoneuroimmunological function needs to be taken into account when measure the behavioral effects of dioscorea.

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References

Araghiniknam M, Chung S, Nelson-White T, Eskelson C and Watson RR (1996) Antioxidant activity of dioscorea and dehydroepiandrosterone (DHEA) in older humans. *Life Sci* **59**:PL147-157.

Armario A, Gil M, Marti J, Pol O and Balasch J (1991) Influence of various acute stressors on the activity of adult male rats in a holeboard and in the forced swim test. *Pharmacol Biochem Behav* **39**:373-377.

Benghuzzi H, Tucci M, Eckie R and Hughes J (2003) The effects of sustained delivery of diosgenin on the adrenal gland of female rats. *Biomed Sci Instrum* **39**:335-340.

Blanchard DC, Blanchard RJ, Tom P and Rodgers RJ (1990) Diazepam changes risk assessment in an anxiety/defense test battery. *Psychopharmacology (Berl)* **101**:511-518.

Bosse R and Di Paolo T (1995) Dopamine and GABAA receptor imbalance after ovariectomy in rats: model of menopause. *J Psychiatry Neurosci* **20**:364-371.

Chen H, Wang C, Chang CT and Wang T (2003) Effects of Taiwanese yam (Dioscorea japonica Thunb var. pseudojaponica Yamamoto) on upper gut function and lipid metabolism in Balb/c mice. *Nutrition* **19**:646-651.

Connor TJ, Song C, Leonard BE, Merali Z and Anisman H (1998) An assessment of the effects of central interleukin-1beta, -2, -6, and tumor necrosis factor-alpha administration on some behavioural, neurochemical, endocrine and immune parameters in the rat. *Neuroscience* **84**:923-933.

Davidson JM (1985) Sexual behavior and its relationship to ovarian hormones in the menopause. *Maturitas* **7**:193-201.

Erb RE, Gomes WR, Randel RD, Estergreen VL, Jr. and Frost OL (1968) Effect of ovariectomy on concentration of progesterone in blood plasma and urinary estrogen excretion rate in the pregnant bovine. *J Dairy Sci* **51**:420-427.

Fernandez-Guasti A, Ferreira A and Picazo O (2001) Diazepam, but not buspirone, induces similar anxiolytic-like actions in lactating and ovariectomized Wistar rats. *Pharmacol Biochem Behav* **70**:85-93.

Frye CA and Walf AA (2004) Estrogen and/or progesterone administered systemically or to the amygdala can have anxiety-, fear-, and pain-reducing effects in ovariectomized rats. *Behav Neurosci* **118**:306-313.

Hanisch UK (2001) Effects of Interleukin-2 and interferons on the nervous system, in *Psychoneuroimmunology* (Ader R, Felten DL and Cohen N eds) pp 585-631, Academic Press, San Diego.

Higdon K, Scott A, Tucci M, Benghuzzi H, Tsao A, Puckett A, Cason Z and Hughes J (2001) The use of estrogen, DHEA, and diosgenin in a sustained delivery setting as a novel treatment approach for osteoporosis in the ovariectomized adult rat model. *Biomed Sci Instrum* **37**:281-286.

Ho YJ, Chang YC, Liu TM, Tai MY, Wong CS and Tsai YF (2000) Striatal glutamate release during novelty exposure-induced hyperactivity in olfactory bulbectomized rats. *Neurosci Lett* **287**:117-120.

Ho YJ, Eichendorff J and Schwarting RK (2002) Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behav Brain Res* **136**:1-12.

Ho YJ, Hsu LS, Wang CF, Hsu WY, Lai TJ, Hsu CC and Tsai YF (2005) Behavioral effects of d-cycloserine in rats: the role of anxiety level. *Brain Res* **1043**:179-185.

Iwu MM, Okunji CO, Ohiaeri GO, Akah P, Corley D and Tempesta MS (1990) Hypoglycaemic activity of dioscoretine from tubers of Dioscorea dumetorum in normal and alloxan diabetic rabbits. *Planta Med* **56**:264-267.

Katase K, Kato T, Hirai Y, Hasumi K and Chen JT (2001) Effects of ipriflavone on bone loss following a bilateral ovariectomy and menopause: a randomized placebo-controlled study. *Calcif Tissue Int* **69**:73-77.

Kim MJ, Kim HN, Kang KS, Baek NI, Kim DK, Kim YS, Jeon BH and Kim SH (2004) Methanol extract of Dioscoreae Rhizoma inhibits pro-inflammatory cytokines and mediators in the synoviocytes of rheumatoid arthritis. *Int Immunopharmacol* **4**:1489-1497.

Koh KB and Lee Y (2004) Reduced anxiety level by therapeutic interventions and cell-mediated immunity in panic disorder patients. *Psychother Psychosom* **73**:286-292.

Lacosta S, Merali Z and Anisman H (1999) Influence of acute and repeated interleukin-2 administration on spatial learning, locomotor activity, exploratory behaviors, and anxiety. *Behav Neurosci* **113**:1030-1041.

Lee SC, Tsai CC, Chen JC, Lin CC, Hu ML and Lu S (2002) The evaluation of reno- and hepatoprotective effects of huai-shan-yao (Rhizome Dioscoreae). *Am J Chin Med* **30**:609-616.

Linzmayer L, Semlitsch HV, Saletu B, Bock G, Saletu-Zyhlarz G, Zoghlami A, Gruber D, Metka M, Huber J, Oettel M, Graser T and Grunberger J (2001) Double-blind, placebo-controlled psychometric studies on the effects of a combined estrogen-progestin regimen versus estrogen alone on performance, mood and personality of menopausal syndrome patients. *Arzneimittelforschung* **51**:238-245.

Marker (1940a) Dioscoreaceae. *J Am Chem Soc* **62**:2542.

Marker RE (1940b) Sterols. CV. The preparation of testosterone and related compounds from sarsasapogenin and diosgenin. *J Am Chem Soc* **62**:2543-2547.

Marker RE, Turner DL and Ulshafer PR (1940) Sterols. CIV. Diosgenin from certain American plants. *J Am Chem Soc* **62**:2542-2543.

Marker RE, Wagner RB, Ulshafer PR and Wittbecker EL (1943) Sterols. CLVII. Sapogenins. LXIX. Isolation and structures of thirteen new steroidal sapogenins. New sources for known sapogenins. *J Am Chem Soc* **65**:1199-1209.

Pellow S, Chopin P, File SE and Briley M (1985) Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* **14**:149-167.

Petitto JM, Huang Z, Hartemink DA and Beck R, Jr. (2002) IL-2/15 receptor-beta gene deletion alters neurobehavioral performance. *Brain Res* **929**:218-225.

Petitto JM, McCarthy DB, Rinker CM, Huang Z and Getty T (1997) Modulation of behavioral and neurochemical measures of forebrain dopamine function in mice by species-specific interleukin-2. *J Neuroimmunol* **73**:183-190.

Porsolt RD, Le Pichon M and Jalfre M (1977) Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**:730-732.

Rosenkranz G, Djerassi C, Yashin R and Pataki J (1951) Cortical hormones from allsteroids; synthesis of cortisone from Reichsteen's compound D. *Nature* **168**:28.

Sagsoz N, Oguzturk O, Bayram M and Kamaci M (2001) Anxiety and depression before and after the menopause. *Arch Gynecol Obstet* **264**:199-202.

Sharkey LC, Holycross BJ, Park S, Shiry LJ, Hoepf TM, McCune SA and Radin MJ (1999) Effect of ovariectomy and estrogen replacement on cardiovascular disease in heart failure-prone SHHF/Mcc- fa cp rats. *J Mol Cell Cardiol* **31**:1527-1537.

Yin J, Kouda K, Tezuka Y, Tran QL, Miyahara T, Chen Y and Kadota S (2003) Steroidal glycosides from the rhizomes of Dioscorea spongiosa. *J Nat Prod* **66**:646-650.

Zalcman S, Murray L, Dyck DG, Greenberg AH and Nance DM (1998) Interleukin-2 and -6 induce behavioral-activating effects in mice. *Brain Res* **811**:111-121.

Zava DT, Dollbaum CM and Blen M (1998) Estrogen and progestin bioactivity of foods, herbs, and spices. *Proc Soc Exp Biol Med* **217**:369-378.

Zimmerberg B and Farley MJ (1993) Sex differences in anxiety behavior in rats: role of gonadal hormones. *Physiol Behav* **54**:1119-1124.

Figure legend

Figure 1. Effects of chronic treatment of dioscorea on the immobility in FS test. Dioscorea was orally administered for 23 days. Number of rats per group is shown in parentheses below each group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared to the data of day-1 session, pared *t*-test. ## $p < 0.01$, compared to the rats treated with identical dosage in sham-operated group. Mean ± SEM are expressed.

Table 1. Time course of the treatment and behavioral test taken in the present study.

Day	1	2-27	28	29	30-50	51	52	53	54-55	56
Treatment	Ovariectomy			Dio	Dio	Dio	Dio	Dio	Dio	Sacrifice
Test			Open field (10 min)	EPM (5 min)		EPM (5 min)	FS day-1 (15 min)	FS day-2 (5 min)		

Ovariectomy was performed on the day 1. Dioscorea (Dio) was administered daily from day 29 to day 55. EPM: elevated plus-maze; FS: forced swim.

Table 2. Effects of ovariectomy on the behavior in the EPM test.

	Sham	**OVX**	
	(n=31)	LA (n=34)	HA (n=34)
OAT	50.6 ± 7.4	56.3 ± 4.6	5.3 ± 1.6$^{\#\#\#}$***
CAT	215.7 ± 9.5	211.2 ± 5.7	270.9 ± 4.2$^{\#\#\#}$***
OAA	6.0 ± 1.1	7.6 ± 1.0	0.7 ± 0.3$^{\#\#\#}$***
CAA	21.2 ± 1.8	21.6 ± 1.3	26.9 ± 1.6$^{\#}$*
TAA	27.2 ± 2.1	29.2 ± 1.8	27.6 ± 1.7

# $p < 0.05$, ### $p < 0.001$, compared with sham-operated group, * $p < 0.05$, *** $p < 0.001$, compared with LA group. Abbreviation: LA: low anxiety; HA: high anxiety; OAT: open arm time; CAT: enclosed arm time; OAA: open arm activity; CAA: enclosed arm activity; TAA: total arm activity. Mean ± SEM are expressed.

Table 3. Effects of dioscorea on the EPM behavior in the sham-operated rats.

		0 mg/kg/day (n=11)	250 mg/kg/day (n=11)	750 mg/kg/day (n=9)
OAT	before	40.4 ± 11.5	57.7 ± 11.9	54.4 ± 16.3
	after	41.5 ± 10.2	58.7 ± 13.0	35.7 ± 9.9
CAT	before	228.9 ± 16.7	206.0 ± 16.3	211.4 ± 17.4
	after	221.7 ± 15.0	174.3 ± 25.5	225.8 ± 13.1
OAA	before	3.9 ± 1.3	7.7 ± 1.7	6.6 ± 2.5
	after	6.2 ± 1.5	8.2 ± 1.6	6.0 ± 2.1
CAA	before	20.5 ± 2.6	22.3 ± 3.9	20.7 ± 3.0
	after	22.6 ± 2.4	18.4 ± 2.6	23.6 ± 1.6
TAA	before	24.4 ± 3.4	30.0 ± 3.6	27.2 ± 4.0
	after	28.7 ± 2.6	26.6 ± 3.1	29.6 ± 2.5

Abbreviation: OAT: open arm time; CAT: enclosed arm time; OAA: open arm activity; CAA: enclosed arm activity; TAA: total arm activity. Data, “before” and “after” 3-week dioscorea treatment, are shown as Mean ± SEM.

Table 4. Effects dioscorea on the behavior in EPM test in OVX rats.

		LA				HA			
		0 mg/kg/day (n=8)	250 mg/kg/day (n=10)	750 mg/kg/day (n=9)	1500 mg/kg/day (n=7)	0 mg/kg/day (n=7)	250 mg/kg/day (n=8)	750 mg/kg/day (n=9)	1500 mg/kg/day (n=10)
OAT	before	59.3 ± 8.9	50.6 ± 6.5	57.5 ± 9.6	59.6 ± 14.5	5.1 ± 3.6	6.0 ± 3.3	6.5 ± 3.4	3.9 ± 2.7
	after	32.3 ± 11.4	23.2 ± 7.1 **	49.6 ± 9.5	37.6 ± 14.2	10.8 ± 5.8	17.2 ± 6.9	33.5 ± 8.4 **	21.3 ± 7.2 *
CAT	before	205.5 ± 9.4	220.8 ± 8.8	209.3 ± 12.5	206.6 ± 16.8	277.0 ± 11.8	269.2 ± 7.5	261.8 ± 9.6	276.3 ± 5.3
	after	228.3 ± 19.4	247.0 ± 14.6	211.1 ± 15.6	224.2 ± 22.4	264.2 ± 10.7	264.9 ± 10.4	239.3 ± 13.9 *	239.6 ± 12.8 *
OAA	before	8.0 ± 0.8	5.2 ± 0.9	9.7 ± 2.8	8.0 ± 2.8	0.6 ± 0.6	0.8 ± 0.5	0.9 ± 0.9	0.4 ± 0.3
	after	6.3 ± 1.9	2.8 ± 0.9 *	7.6 ± 1.9	5.4 ± 2.3	1.1 ± 0.9	2.5 ± 1.2	3.9 ± 1.3 **	1.8 ± 0.7
CAA	before	21.8 ± 3.4	19.5 ± 1.3	24.0 ± 2.8	21.3 ± 3.4	27.1 ± 3.3	23.1 ± 3.4	28.4 ± 3.4	28.5 ± 3.1
	after	20.5 ± 3.5	22.1 ± 3.0	21.3 ± 2.4	21.1 ± 3.5	20.6 ± 1.9	18.1 ± 2.8	19.9 ± 2.6 *	24.9 ± 2.8
TAA	before	29.8 ± 3.5	24.7 ± 1.7	33.7 ± 4.2	29.3 ± 5.3	27.7 ± 3.5	23.9 ± 3.5	29.3 ± 3.5	28.9 ± 3.0
	after	26.8 ± 4.0	24.9 ± 3.1	28.9 ± 3.0	26.6 ± 3.5	21.7 ± 2.0	20.6 ± 2.8	23.8 ± 2.5	26.7 ± 2.7

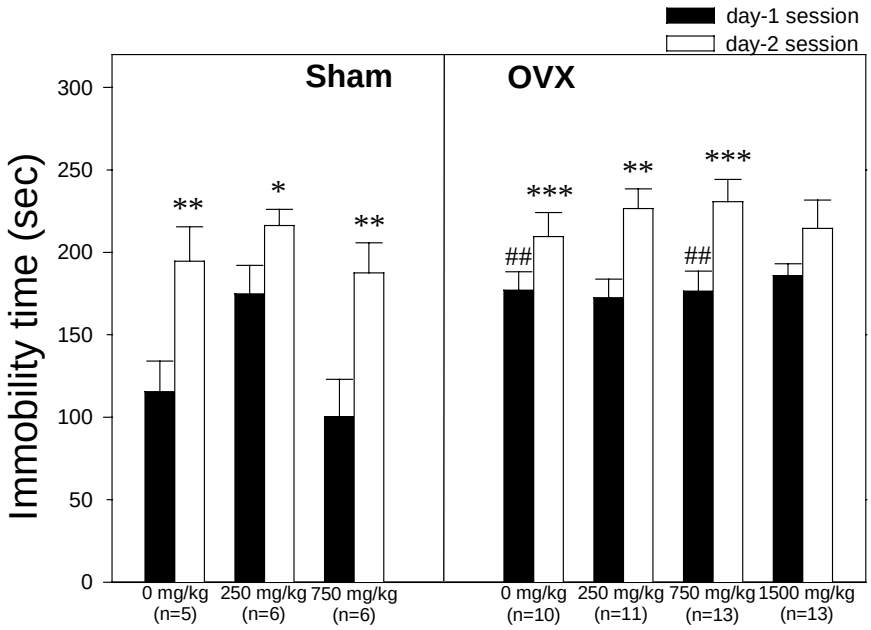
LA: low anxiety; HA: high anxiety. * $p < 0.05$, ** $p < 0.01$, paired *t*-test, compared with the data before dioscorea treatment. Abbreviation: OAT: open arm time; CAT: enclosed arm time; OAA: open arm activity; CAA: enclosed arm activity; TAA: total arm activity. Data, “before” and “after” 3-week dioscorea treatment, are shown as Mean ± SEM.

Table 5. Effects of dioscorea on the level of IL-2 in the brain.

	Sham			OVX							
				LA				HA			
	0 mg/kg/day (n=10)	250 mg/kg/day (n=11)	750 mg/kg/day (n=9)	0 mg/kg/day (n=8)	250 mg/kg/day (n=10)	750 mg/kg/day (n=9)	1500 mg/kg/day (n=7)	0 mg/kg/day (n=7)	250 mg/kg/day (n=7-8)	750 mg/kg/day (n=9)	1500 mg/kg/day (n=10)
PFC	2.4 ± 0.2	2.7 ± 0.3	2.4 ± 0.2	1.7 ± 0.2#	2.5 ± 0.1*	2.2 ± 0.3	1.8 ± 0.3	1.9 ± 0.2	2.1 ± 0.2	2.3 ± 0.3	2.2 ± 0.2
CORT	1.9 ± 0.4	1.8 ± 0.3	1.3 ± 0.2	1.6 ± 0.2	1.8 ± 0.3	2.1 ± 0.4	1.7 ± 0.2	2.7 ± 0.6	1.5 ± 0.2*	1.5 ± 0.2**	1.4 ± 0.2**

LA: low anxiety; HA: high anxiety. # $p < 0.05$, compared with the rats treated by 0 mg/kg/day in sham-operated group; * $p < 0.05$, ** $p < 0.01$, compared with the rats treated by 0 mg/kg/day in same anxiety category. Data are expressed as Mean ± SEM; unit is pg/μg protein.

Fig. 1


day-1 session
day-2 session
Sham
OVX
Immobility time (sec)
300
250
200
150
100
50
0
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##
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0 mg/kg (n=5)
250 mg/kg (n=6)
750 mg/kg (n=6)
0 mg/kg (n=10)
250 mg/kg (n=11)
750 mg/kg (n=13)
1500 mg/kg (n=13)