Sedative Effect of *Sophora flavescens* and Matrine

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**Abstract**

The present study investigated the sedative effects of *Sophora flavescens* (SF) and its bioactive compound, matrine through performing locomotor activity test and the electroencephalography (EEG) analysis in the rat. The underlying neural mechanism of their beneficial effects was determined by assessing c-Fos immunoreactivity and serotonin (5-HT) in the brain utilizing immunohistochemical method and enzyme-linked immunosorbent assay. The results showed that SF and matrine administration had an effect on normalization of caffeine-induced hyperactivity and promoting a shift toward non-rapid eye movement (NREM) sleep. c-Fos-immunoreactivity and 5-HT level in the ventrolateral preoptic nucleus (VLPO), a sleep promoting region, were increased in the both SF and matrine-injected groups. In conclusion, SF and its bioactive compound, matrine alleviated caffeine-induced hyperactivity and promoted NREM sleep by activating VLPO neurons and modulating serotonergic transmission. It is suggested that SF might be a useful natural alternatives for hypnotic medicine.

**Key Words:** *Sophora flavescens*, Matrine, Serotonin, Electroencephalography, Locomotor, Sedative effect

**INTRODUCTION**

Sleep is an essential component for assessing health-related quality of life. Sleep disturbance causes dysregulation of homeostasis and increases vulnerability to inflammation-related diseases (Irwin et al., 2016) and chronic diseases (Lima et al., 2012). Benzodiazepines are the most commonly prescribed medications for treatment of insomnia and have been reported to be associated with adverse outcomes such as mood disorder, lung disease (Kroll et al., 2016) and cognitive deficit (Pariente et al., 2016). Regarding these side effects, importance of developing alternative natural medicines has been growing.

*Sophora* is a genus in the Fabaceae family and the root of *Sophora flavescens* (SF), which is known as Kushen, has been used in traditional remedy to treat fever, dysentery and pain (He et al., 2015). SF has been reported to have an anti-infection (Yanju et al., 2014), an anti-rotaviral (Alfajar et al., 2014), a recovery from injury (Tanabe et al., 2016) and whitening effect (Shin et al., 2013). Matrine is alkaloids which is one of the bioactive components in SF and is reported to have pharmacological effects on cardiac diseases (He et al., 2015), inflammation (Shao et al., 2013) and tumor (Liu et al., 2014). Neither SF nor matrine have been reported to have sedative effect yet, however, accumulating evidence of correlation between sleep disturbance and inflammatory disease (Irwin et al., 2016) suggests that anti-inflammatory efficacy of SF and matrine may contribute to sedative effect.

The ventrolateral preoptic nucleus (VLPO) located in the anterior hypothalamus acts as a sleep-promoting center and molecular targets of hypnotic medication (Ferre, 2008). Sleep active neurons have been identified by the immunohistochemical detection of the Fos protein during sleeping. Therefore, it is possible that activation of the VLPO region indicates initiation of sleep-state.

It has been known that neurons in the VLPO contain serotonin (5-HT) and its receptors, which is one of the most important neurotransmitters for its role in regulating sleep-wake homeostasis (Melancon et al., 2014; Whitney et al., 2016). Sleep-wake state is assessed by evaluating shifts of the electroencephalogram (EEG) frequencies. A continuous transition from wake period to non-rapid eye movement (NREM) followed by rapid eye movement (REM) occurred (Irwin, 2015). Wakefulness is identified by the predominance of high
frequency and low amplitude EEG. NREM is scored based on the presence of spindles interspersed with slow waves in the EEG. EEG power during REM was significantly reduced in lower frequency θ-wave and increased in the range of δ-wave activity.

The purpose of this study was to investigate the sedative effects of SF and its bioactive compound, matrine through performing locomotor activity test and EEG analysis. The underlying neural mechanism of the beneficial effect was determined by measuring c-Fos immunoreactivity and 5-HT in the brain utilizing immunohistochemical method and enzyme linked immunosorbent assay.

MATERIALS AND METHODS

Animals

All the experimental procedures performed on the animals were conducted with the approval of the Ethics Committee of the Kyung Hee University (KHUAP(SE)-13-041; Seoul, Korea) and in accordance with the US National Institutes of Health (Guide for the Care and Use of Laboratory Animals, 8th edition, revised 2011). Male Sprague-Dawley rats (Samtaco Animal Corp., Gyeonggi, Korea) aged 7 weeks (280-320 g) were housed in an air-conditioned room kept at a temperature of 20-25°C and a humidity of 45-65%, with a 12:12 h light/dark cycle (lights-on at 8 a.m.). Animals had access to water and food ad libitum and were acclimatized for 7 days prior to experiments.

Preparations of methanol extract of SF and matrine

SF was purchased from Dongwoodang Pharmacy Co., Ltd (Gyungbuk, Korea). A voucher specimen of SF has been deposited at the herbarium located at the College of Korean Medicine, Kyung Hee University (Reference No. KH-SFLM01). The dried SF (200 g) was extracted with 10-fold volume of 70% of methanol in the ultrasound bath and filtered through Whatman filter paper (Maidstone, UK). The extracts were concentrated with a rotary evaporator (Eyela, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) under reduced pressure (~760 mmHg) yielding 22.8 w/w%. Matrine was purchased from Eon MED CHEM Co., Ltd (Jinan, China).

Administration of SF and matrine

Methanol extract of SF was dissolved in 0.9% saline at a concentration of 400 mg/ml (SF400) or 800 mg/ml (SF800) then administered per os (p.o.) in a volume of 10 ml/1 kg of body weight. Matrine was dissolved in 0.9% saline at a concentration 30 mg/ml (M30) or 100 mg/ml (M100) then injected intraperitoneally (i.p.) in a volume of 1 ml/1 kg of body weight.

Measurement of serotonin (5-HT) level

After EEG recordings, the animals were deeply anesthetized with sodium pentobarbital (80 mg/kg, administered i.p.) and the brains were immediately removed and coronally sectioned by using rodent brain matrix (ASI instruments Inc., MI, USA). The cortex and VLPO regions of the brain were punched out on a cold plate and stored at -70°C until the assay. The obtained tissue was homogenized and incubated in ice cold protein extraction solution (iNtRON Biotechnology, Inc., Gyeonggi, Korea) for 30 min and centrifuged (10,000×g at 4°C for 5 min). Supernatant was transferred to a fresh tube and 5-HT concentration in duplicate aliquots was assessed by enzyme linked immunosorbent assay (ELISA) kit according to the manufacturer’s instructions (Labor Diagnostika Nord, Inc., Minneapolis, MN, USA).

c-Fos immunohistochemistry

After 24 hours later of SF or matrine administration, all rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, i.p.). The rat brains were removed after transcardial perfusion with 4% solution of formaldehyde (Sigma-Aldrich Co.), then post-fixed in the same fixative for 24 hours, and placed in PBS containing 20% of sucrose for 72 hours. Serial coronal sections were cut into 30 μm thickness by using a cryostat microtome (CM1850UV, Leica Microsystems Inc., Wetzlar, Germany). The sections were stored at -20°C and these slices were histochemically processed as free-floating sections.
The brain sections were washed three times in PBS containing 0.2% Triton X-100 (PBST). The primary rabbit monoclonal antibodies against c-Fos were used (diluted 1:2000 in PBST with 10% v/v normal goat serum, Santa Cruz Biotechnology, Inc., TX, USA) and the sections were incubated for 72 hours at 4°C with constant agitation. After rinsing in PBST, the sections were incubated in biotinylated goat anti-rabbit (Vector Laboratories, Inc., Burlingame, CA, USA) diluted 1:200 in PBST with 2% v/v normal goat serum for 2 hours at room temperature. The sections were then incubated in the avidin-biotin-peroxidase complex reagent (Vector Laboratories) for 2 hours at room temperature. Following further rinsing with PBST, the tissues were developed using diaminobenzadine substrate kit (Vector Laboratories, Inc., Burlingame, CA, USA) diluted 1:200 in PBST with 2% v/v normal goat serum for 2 hours at room temperature. The numbers of immunopositive neurons were regarded as statistically significant. *p<0.05 vs caffeine treated group; one-way ANOVA.

RESULTS

Effects of SF and matrine on caffeine-induced hyperactivity

Locomotor activity was measured to investigate sedative effect of SF and matrine on caffeine-induced hyperactivity. Traveled distance was recorded and analyzed during latter 1 hour (Fig. 1). SF 800-treated group showed significant reduction on locomotor activity [F(3,18)=5.510, p<0.05, Fig.1A] and matrine-administered group had a dose-dependent tendency to decrease movements [F(3,27)=1.990, p=0.142, Fig.1B].

Effects of SF and matrine on EEG

Effects of SF and matrine on EEG sleep architecture and profile were presented in Fig. 2. The EEG signals in rats were recorded for 12 hours after oral SF and matrine treatment (SF; 400 and 800 mg/kg, matrine; 30 and 100 mg/kg) at 08:00 PM. SF (800 mg/kg) significantly decreased wake time and increased NREM time [F(2,17)=4.810, *p<0.05 and F(2,17)=4.467, **p<0.05, Fig. 2A, 2C]. No significant change in REM sleep was observed, but slight increase was shown in dose-dependent manner [F(2,17)=0.585, Fig. 2A, 2B]. Matrine (30 mg/kg) significantly decreased wake time and increased NREM time [F(2,17)=4.810, *p<0.05 and F(2,17)=4.467, **p<0.01 vs saline treated group; one-way ANOVA].

Fig. 1. The effect of (A) SF and (B) matrine on caffeine-induced hyperactivity. Each column represents the mean ± SEM. *p<0.05 vs saline treated group, #p<0.05 vs caffeine treated group; one-way ANOVA.

Fig. 2. The effect of SF and matrine on sleep architecture. Changes in the percentage of wake (A, D), REM sleep (B, E) and NREM sleep (C, F) during dark phase were shown in the SF and matrine treated groups. The data represent the mean ± SEM of percent time spent in sleep-wake state. *p<0.05 vs saline treated group; one-way ANOVA.

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DISCUSSION

The findings of the current research demonstrated the sedative efficacy of SF and its bioactive compound, matrine-type alkaloids have sedative efficacy. Pretreatment of SF and matrine prior to caffeine administration alleviated caffeine-induced hyperactivity. Administration of SF and matrine significantly reduced total wake time and increased NREM time. 5-HT level in the cortex and VLPO and c-Fos expression in the VLPO were considerably increased in the SF and matrine group. The data showed the first experimental evidence that *Sophora flavescens* has sedative efficacy suggesting SF and matrine as novel natural medications to enhance sleep by modulating serotonergic system and activating sleep-promoting region in the brain.

It has been reported that 1g of SF contains 1.21 mg of matrine (Li and Wang, 2004). In accordance with this report, it is assumed that 400 mg of SF extracts contain approximate 22 mg of matrine. Therefore, two matrine doses, 30 mg/ml and 100 mg/ml were chosen in this study.

Caffeine increased locomotor activity and wake time and proportionally diminished both NREM and REM time (Okuro et al., 2010) and caffeine at a dose of 10 mg/kg caused insomnia in the rat (Paterson et al., 2007). Caffeine disrupts sleep by antagonizing adenosine A1A receptors in the VLPO thereby reduces inhibitory transmission to the tuberomammillary nucleus (TMN) which is responsible for wakefulness-promotion (Ferre, 2008). It was reported that attenuation of locomotor activity is innovative approach to assess sedative effect of herbal extracts (Klein et al., 2014). The present study showed that SF and matrine administration had an effect on normalization of caffeine (10 mg/kg)-induced hyperactivity indicating that SF and matrine can be candidates to treat insomnia. 5-HT depletion caused significant increment of motor activity and modulation of sleep-wake cycle (Solarewicz et al., 2015). Thus, it is suggested that locomotor-reducing ability of SF and matrine may contribute to sleep promotion through increasing 5-HT release. Previous studies have reported that administration of extracts from natural herbal medicine, which exhibited hypnotic effect, altered total sleep time and NREM rather than REM sleep (Abdollahnejad et al., 2016; Hajjaghaee et al., 2016). In accordance with these studies, the present research showed that SF and matrine at a dose of 800 mg/kg and 30 mg/kg, respectively, reduced wake time considerably and SF (800 mg/kg) promoted a significant shift toward NREM sleep.

The central serotonergic systems have been implicated in promoting wakefulness (Monti, 2011), but the exact role on sleep-wake system is still controversial. Extracellular 5-HT release during sleep deprivation was reduced (Bjorvatn et al., 2002) or increased (Lopez-Rodriguez et al., 2003). Tryptophan, the precursor of 5-HT, was reported to improve sleep (Bravo et al., 2013; Melancon et al., 2014). Another study suggested that 5-HT depletion-induced sleep disturbance occurs only in condition under hypothermia (Murray et al., 2015). In the present study, 5-HT level in the VLPO, a sleep promoting region, and in the cortex was increased in the both SF and matrine-injected groups. There are two types of VLPO neurons which differently respond to 5-HT. 5-HT suppresses input to type 1 neurons which have 5-HT 1, 2, 4, 7 receptors. Input to type 2 neurons containing 5-HT 2, 4, 7 receptors, however, is enhanced by 5-HT. Although 2 neurons are inversely influenced by 5-TH, they promotes sleep by involving initiation and maintenance of sleep, respectively (Sangare et al., 2016). It was also reported that injection of 5-HT receptor 1A agonist increases wake time (Monti and Jantos, 2003). Accumulating evidences and the present results drives that increment 5-HT release.
in VLPO may promote sleep through diverse 5-HT receptors located in type 1 and 2 neurons.

Fos protein, a marker for postsynaptic activation, is expressed in the neurons of the VLPO only during sleep-phase (Sherin et al., 1996). In agreement with this result, it was observed that treatment of matrine caused significantly increased expression of c-Fos-immunoreactivity in the VLPO and SF-treated group showed tendency to increase c-Fos expression.

SF800 group showed significant changes in locomotor activity, wake-sleep state and 5-HT concentration, whereas c-Fos immunoreactivity was slightly increased. Notable changes in 5-HT level and c-Fos expression followed by enhanced total wake time were observed in the Matrine 100 mg/kg-treated group. Taken together, it is assumed that increased release of 5-HT in the VLPO and cortex is sufficient to induce sedative behavioral changes.

It has been shown that the VLPO cells contain a variety of neurotransmitters including GABA and 5-HT. The role of these 5-HT containing cells in regulating the sleep behaviors is clearly not known, but it may be possible that SF and matrine may activate the central serotonergic cells and increase release in the VLPO. In turn, activated c-Fos immunoreactive cells, evidenced by increased c-Fos immunoreactivity, play an important role in facilitating sleep initiation and maintenance as well as inhibiting activation of neurons which involved in arousal. Further studies examining interaction of c-Fos immunoreactive cells and 5-HT receptors in the VLPO will interpret the exact neural mechanisms involved in sleep promotion.

In conclusion, SF and its bioactive compound, matrine alleviated caffeine-induced hyperactivity, increased NREM and decreased wake time by activation of sleep-promoting center, VLPO, and modulating serotonergic transmission. It is suggested that SF might be a useful natural alternatives for hypnotic medicine.

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