Natural Product Sciences
15(4) : 213-221 (2009)

Methanol Extract of Longanae Arillus Regulates Sleep Architecture and EEG Power Spectra in Restraint-Stressed Rats

Yuan Ma[1], Jae Soon Eun[2], Kwang Seung Lee[3], Eun-Sil Lee[3], Chung-Soo Kim[4], Bang-Yeon Hwang[4], and Ki-Wan Oh[4,*]

[1]*Beijing Institute of Pharmacology & Toxicology, Beijing,100850, China.*
[2]*College of Pharmacy, Woosuk University, Samrye 565-701, Republic of Korea*
[3]*Research Institute of Nonghyup Korea Ginseng, Jeungpyung 368-811, Republic of Korea*
[4]*College of Pharmacy, Chungbuk National University, Cheongju 361-763, Republic of Korea*

Abstract – Longanae Arillus (the rind of fruits of *Dimocarpus longan*) has been consumed for the treatment of insomnia and anxiety in Asia. To provide further scientific basis to traditional uses of this fruit on insomnia, we evaluated the effects of methanol extract of Longanae Arillus (MELA) on the alteration of sleep architecture and electroencephalogram (EEG) power spectra in acutely and chronically restraint-stressed rats. Following post-surgical recovery, Polygraphic signs of sleep-wake activities were recorded for 24 h after MELA administration in rats. Rats in the acute stress and chronic stress were administered with MELA for 10 days. On the 8th, 9th and 10th day of MELA administration, the rats were stressed for 3 h once per day. On the 10th day and 1 h after MELA administration, the rats were stressed once for 22 h in the chronic stress group. Acute and chronic stress induced alternations in cortex EEG recordings during non-rapid eye movement (NREM), rapid eye movement (REM) sleep and wakefulness. MELA shortened the total and REM sleep and increased the wakefulness in night time recording without changing daytime recordings. Chronic stress increased wakefulness and REM sleep, decreased total and NREM sleep in the daytime recording, and increased REM and decreased NREM sleep without changing total sleep and wakefulness in night time recording. These findings suggest that MELA ameliorated the alterations in REM and NREM sleep of acutely and chronically stressed rats via modulation of cortical α-, θ- and δ- wave activity.

Keywords – Longanae Arillus, Sleep architecture, Electroencephalogram (EEG) power spectra, Restraint-stress, Non-rapid eye movement (NREM), Rapid eye movement (REM)

Introduction

Longanae Arillus (the rind of fruits of *Dimocarpus longan,* Sapindaceae) is a subtropical fruit widely grown in China and South East Asia (Okuyama *et al*., 1999). It is consumed for the treatment of insomnia and anxiety in Asia (Sun *et al*., 2007). Longan contains many pharmacologically active components that have antioxidant, anticarcinogenic, and vasorelaxing activities as well as anxiolytic activity (Okuyama *et al*., 1999; Festa *et al*., 2001; Kawada *et al*., 2001; Yilmaz *et al*., 2004; Rangkadilok *et al*., 2005). Longan fruit extract may therefore be a new source of dietary antioxidants or anxiolytics. In addition, we reported that longan augments pentobarbital-induced sleep behaviors (Ma *et al*., 2009a). However, there are few pharmacological studies of longan in insomnia, despite its use as an herbal medicine (Rangkadilok *et al*., 2007).

Growing evidence indicates a close relationship between the stress response and the resultant sleep pattern (Bodosi *et al*., 2000; Bonnet *et al*., 2000). This notable alternation of sleep after stress suggests the participation of neurotransmitters and hormones (Gonzalez *et al*., 1995; Vazquez-Palacios *et al*., 2004). Chronic immobilization stress produces sleep disturbances such as a decrease in active waking, non-rapid eye movement (NREM) and rapid eye movement (REM) sleep, a blunting of the sleep-wake cycle, and a decrease in REM sleep (Kant *et al*., 1995). This sleep disturbance is one of the deleterious effects that stress has on the central nervous systems (CNS) (Rampin *et al*., 1991). We therefore tested whether methanol extract of Longanae Arillus (MELA) regulates the sleep architecture and electroencephalogram (EEG) power spectra in acutely and chronically restraint-stressed rats.

*Author for correspondence
Tel: +82-43-261-2827; E-mail: kiwan@chungbuk.ac.kr

Experimental

Preparation of methanol extract – The air-dried Longanae Arillus (the rind of fruits) was purchased from an oriental market in Korea and extracted at 50 °C for 48 h with 70% MeOH. The extract was filtered and concentrated using a rotary vacuum evaporator followed by lyophilization. Finally, MELA was freeze-dried and the remaining supernatants were evaporated and used as the test samples. The yield of MELA was about 12.6% (w/w) (Rangkadilok *et al*., 2005; Rangkadilok *et al*., 2007). Dried extract was dissolved in saline before use.

Experimental animals – Adult male Wistar rats (Samtako, Osan, Korea), weighing 250 - 350 g, were maintained, and all experiments were conducted, according to the Guide for the Care and Use of Laboratory Animals (National Academic Press, Washington, DC, 1996). The rats were housed individually with food and water provided *ad libitum* under an artificial 12 h light/dark cycle (lights on at 7:00) and at a constant temperature (22 ± 2 °C). The rats were housed in the departmental holding room for 1 week before testing.

Surgery – Each rat was implanted with a transmitter (Data Sciences International TA11CTA-F40, MN, USA) for recording EEG and activity via telemetry as described previously (Sanford *et al*., 2006)). The body of the transmitter was implanted subcutaneously off the midline and posterior to the scapula and was attached to the skin with 3 sutures for stabilization. Leads from the transmitter were led subcutaneously to the skull and the bare ends were placed in contact with the dura through holes that were made in the skull (A: 2.0 [Bregma], L: 1.5; P: 7.0 [Bregma], L: 1.5 contra-lateral). The electrodes were anchored to the skull with screws and dental cement. All surgical procedures were performed stereotaxically under aseptic conditions. Surgical anesthesia was achieved with pentobarbital (50 mg/kg, ip) and all efforts were made to minimize the suffering of the animals.

Experimental procedure – Stress procedures were approved and monitored by the ethical committee of Chungbuk National University. Following 7 days post-surgical recovery, rats were divided into control (non-stress), acute stress, and chronic stress groups, with 8 rats in each group; the MELA-treated groups were also divided into acute stress and chronic stress groups, with 8 rats in each group. MELA was dissolved in saline and administered 60 min before the application of the restraint stress. Rats in the acute stress MELA group were fed with MELA for 10 days. On the 8th, 9th and 10th day of MELA feeding, the rats were stressed for 3 h once per day. In the chronic stress group, rats were fed with MELA for 10 days. On the 10th day and 1 h after MELA feeding, the rats were stressed once for 22 h. One hour after ending the stress procedure on the 10th day, sleeping behavior and EEG data was continuously monitored for 24 h (12 h day and 12 h night recording). The stress was produced by restraining the animals inside an adjustable acrylic hemi-cylindrical plastic box (7.5-cm diameter, 15-cm long).

Data collection – Telemetric recording of cortical EEG and activity was conducted using procedures similar to previous reports (Ma *et al*., 2009). For the EEG signal, the gain of transmitters was set at –0.5/+0.5 volts per/units × 2 and the raw signals generated from the transmitter were in the range of 0.5-20.0 Hz. The signals were processed by a Data Sciences International analog converter and routed to an AD converter (Eagle PC30, USA) housed in a PC class computer. The AD converter digitized the EEG and activity signals at 128 Hz. The digitized data were transferred to the computer and displayed graphically by the *SleepSign* program on the computer monitor. An on-line fast Fourier transformation (FFT) was performed on the EEG data at 2-sec intervals during data acquisition (256 samples) after a Hanning window treatment. The FFT analysis generated power density values from 0.0 to 20.0 Hz at a resolution of 0.5 Hz. The FFT data were further averaged in the range of 0 to 20 Hz for every 10 sec. The sleep data and FFT results were saved to the hard disk every 10 sec for additional off-line analysis. Movement of the animal in relation to the telemetry receiver generated transistor-transistor logic (TTL) pulses that were collected and counted as a measure of activity. MELA was administered 10 min before the EEG recording. Recording began at 7:00 am for 24 h.

Data analysis in sleep architecture and EEG power spectra – The amount of time in wakefulness, NREM, and REM sleep were determined from the digitized data at 10 sec intervals using professional animal sleep analysis software *SleepSign 2.1* (KISSEI Comtec Co Ltd, Matsumoto, Japan). Briefly, the software discriminates wakefulness as high-frequency, low-amplitude EEG. NREM was scored based on the presence of spindles interspersed with slow waves in the EEG. EEG power during REM is significantly reduced in lower frequency δ-wave (0.75 - 4.0 Hz) and increased in the range of θ-wave activity (5.0 - 9.0 Hz, peak at 7.5 Hz). The time spent (min) in NREM, REM, total sleep time (NREM + REM) and numbers of sleep-wake cycle were processed to obtain 12 h period totals for each rat. We further calculated the time of each recording spent in each sleep-

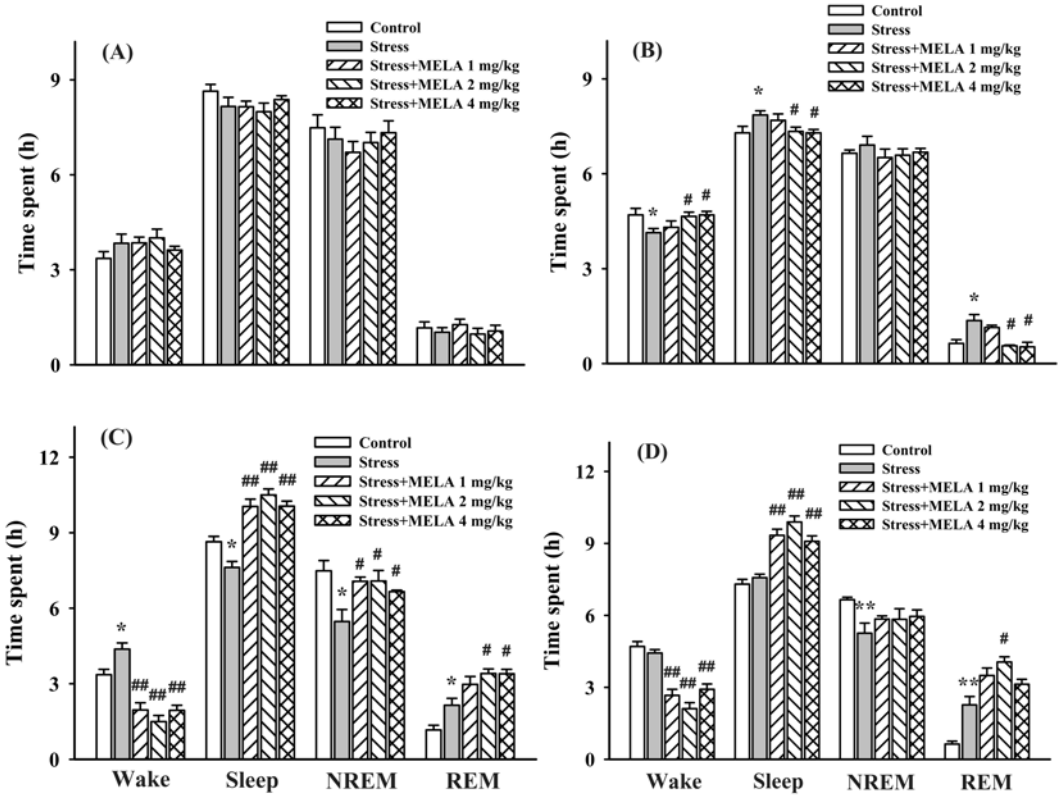

Fig. 1. Effects of MELA on sleep architecture in acutely and chronically stressed rats. The data represent mean ± SE of time spent in sleep-wake state (Wake, Sleep (total sleep), NREM sleep, REM sleep). (A): Day time sleep architecture in acutely stressed rats. (B): Night time sleep architecture in acutely stressed rats. (C): Day time sleep architecture in chronically stressed rats; (D): Night time sleep architecture in chronically stressed rats. *P < 0.05; **P < 0.01, significantly different from control group; $^{\#}$P < 0.05; $^{\#\#}$P < 0.01, significantly different from stress group.

wake state (wake, NREM, REM). The absolute EEG power during wakefulness, NREM, and REM were calculated in 0.5 Hz bins from 0.5 to 20 Hz for the entire 12 h reading of each recording process. EEG power density in 3 selected frequency bands for wakefulness, NREM, and REM, δ-wave (0.75-4.5 Hz), θ-wave (5.0-9.0 Hz), and α-wave (8.0-13.0 Hz)) were subsequently evaluated.

Statistical analysis – All statistical analyses were conducted using SigmaStat software. Analysis of variance (ANOVA) procedures were used in the data analysis, followed by post hoc comparisons using the Tukey test.

Results and Discussion

Fig. 1 shows the effect of MELA on the sleep architecture of acutely and chronically stressed rats. The time spent in wakefulness, total sleep, and REM sleep for acutely stressed rats at night time were significantly different (Wakefulness [$F(4,35) = 3.25$, $p < 0.05$]; Total sleep [$F(4,35) = 3.35$, $p < 0.05$]; REM sleep [$F(4,35) = 3.42$, $p < 0.05$]). Chronic stress significantly changed time spent in wakefulness, total sleep, NREM, and REM sleep during both day and night (day time recording: Wakefulness [$F(4,35) = 4.82$, $p < 0.01$]; Total sleep [$F(4,35) = 3.99$, $p < 0.01$]; NREM sleep [$F(4,35) = 3.55$, $p < 0.05$]; REM sleep [$F(4,35) = 3.71$, $p < 0.05$], night time recording: Wakefulness [$F(4,35) = 4.27$, $p < 0.01$]; Total sleep [$F(4,35) = 4.25$, $p < 0.01$]; NREM sleep [$F(4,35) = 2.90$, $p < 0.05$]; REM sleep [$F(4,35) = 4.81$, $p < 0.01$]). Post hoc comparisons revealed that acute stress decreased wakefulness and increased total and REM sleep at night but did not change daytime recordings. MELA (2 and 4 mg/kg) shortened total and REM sleep and increased wakefulness at night ($p < 0.05$, $p < 0.01$, Fig. 1A, B). Chronic stress increased wakefulness and decreased total sleep during the day but didn't change wakefulness or total sleep at night. Chronic stress increased REM and decreased NREM sleep in both day and night ($p < 0.05$, $p < 0.01$, Fig. 1C, D). MELA (1, 2, and 4 mg/kg) significantly restored total sleep and reduced wakefulness in chronically stressed rats, and

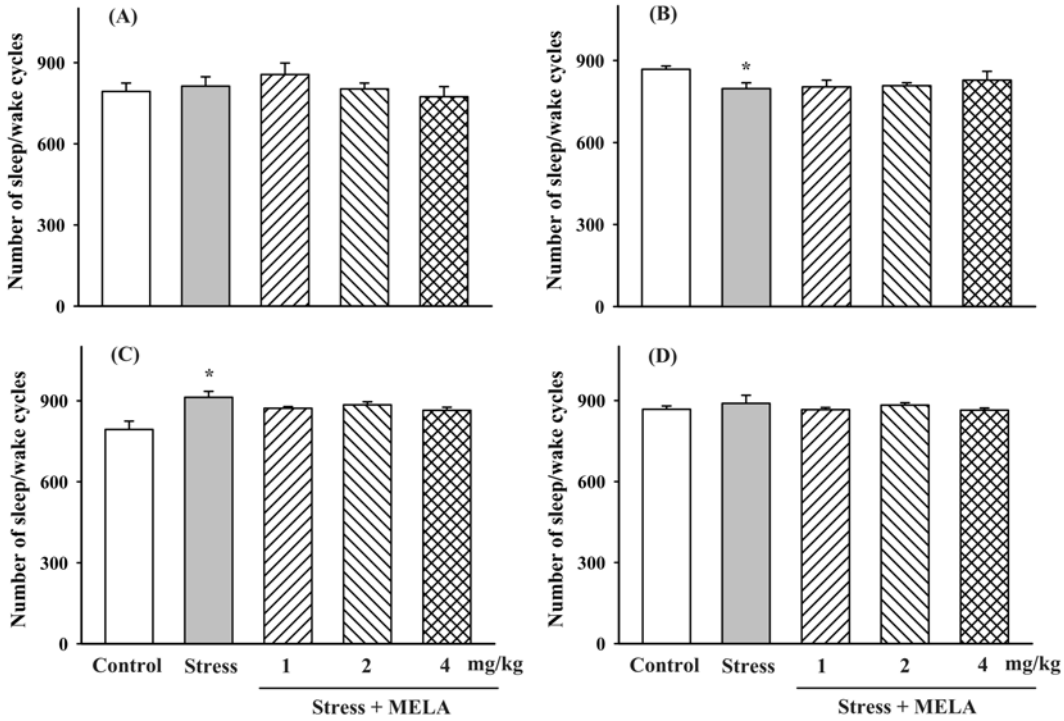

Fig. 2. Effects of MELA on number of sleep/wake changes. The data represent mean ± SE of sleep/wake change numbers. (A): Day time sleep/wake changes in acutely stressed rats. (B): Night time sleep/wake changes in acutely stressed rats. (C): Day time sleep/wake changes in chronically stressed rats. (D): Night time sleep/wake changes in chronically stressed rats. *P < 0.05, significantly different from control group.

increased NREM and REM sleep ($p < 0.05$, $p < 0.01$, Fig. 1C, D).

Fig. 2 presents the effect of MELA on sleep/wake changes in acutely and chronically stressed rats. Acutely stressed rats showed sleep/wake changes at night [$F(4,35) = 3.15$, $p < 0.05$] and chronically stressed rats during the day [$F(4,35) = 3.22$, $p < 0.05$]. Acute stress did not change the number of sleep-wake cycles during the day but reduced them at night ($p < 0.05$, Fig. 2A, B). Chronic stress increased the number of sleep-wake cycles during the night ($p < 0.05$, Fig. 2C). MELA did not influence sleep/wake changes in either stress group.

Fig. 3 shows the effect of MELA on EEG power density during NREM sleep. Chronic stress significantly changed δ-wave, θ-wave and α-wave power density during the day (δ-wave [$F(4,35) = 4.54$, $p < 0.01$]; θ-wave [$F(4,35) = 3.97$, $p < 0.01$]; α-wave [$F(4,35) = 3.35$, $p < 0.05$]) and δ-wave, α-wave power at night δ-wave [$F(4,35) = 4.26$, $p < 0.01$]; α-wave [$F(4,35) = 3.52$, $p < 0.05$]). Acute stress and MELA did not affect δ-, θ- and α-wave power density. Chronic stress increased the day time θ-wave, but decreased the night time α-wave power density ($p < 0.05$, $p < 0.01$, Fig. 3C, D). MELA (1, 2 and 4 mg/kg) increased day time and night time δ-wave power density and decreased the day time α- and θ-wave power density ($p < 0.05$, Fig. 3C, D).

Fig. 4 shows the effect of MELA on EEG power density during REM sleep. Acute stress changed δ-wave, θ-wave, and α-wave power density at night (δ-wave [$F(4,35) = 3.99$, $p < 0.01$]; θ-wave [$F(4,35) = 3.33$, $p < 0.05$]; α-wave [$F(4,35) = 4.53$, $p < 0.01$]), and chronic stress changed θ-wave and α-wave power density during the day and night (day time recording: θ-wave [$F(4,35) = 3.17$, $p < 0.05$]; α-wave [$F(4,35) = 4.55$, $p < 0.01$], night time recording: θ-wave [$F(4,35) = 3.43$, $p < 0.05$]; α-wave [$F(4,35) = 4.23$, $p < 0.01$]). Acute stress increased night time δ-wave and decreased α-wave power density ($p < 0.05$, Fig. 4A,B). MELA (2 and 4 mg/kg) significantly decreased night time δ-wave and increased α-wave power density ($p < 0.05$, $p < 0.01$, Fig. 4B), and 4 mg/kg decreased θ-wave power density ($p < 0.05$, Fig. 4B). Chronic stress increased the day and night θ-wave power density and decreased the α-wave power density only at night ($p < 0.05$, $p < 0.01$, Fig. 4C, D). MELA decreased the day time θ-wave power density and increased day and night α-wave power density ($p < 0.05$, $p < 0.01$, Fig. 4C, D).

Fig. 5 shows the effect of MELA on EEG power

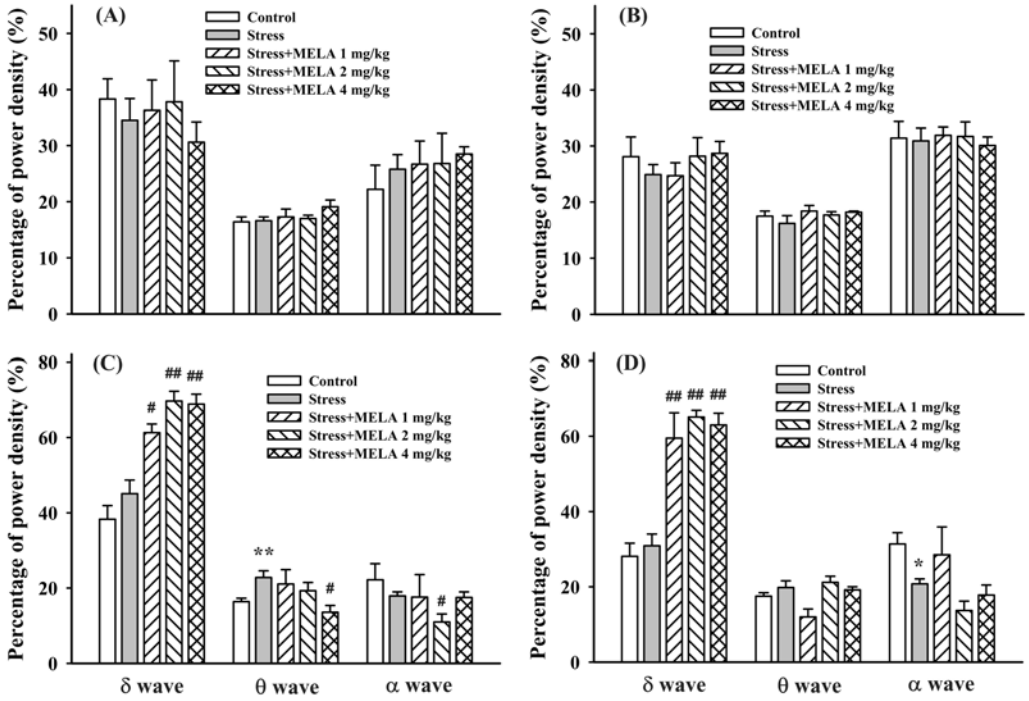

Fig. 3. Effects of MELA on EEG power density during NREM sleep. The data represent mean ± SE of EEG power density in δ-wave, θ-wave, and α-wave bands during NREM sleep. (A): Day time EEG power density during NREM sleep in acutely stressed rats. (B): Night time EEG power density during NREM sleep in acutely stressed rats; (C): Day time EEG power density during NREM sleep in chronically stressed rats. (D): Night time EEG power density during NREM sleep in chronically stressed rats. *P < 0.05; **P < 0.01, significantly different from control group; #P < 0.05; ##P < 0.01, significantly different from stress group.

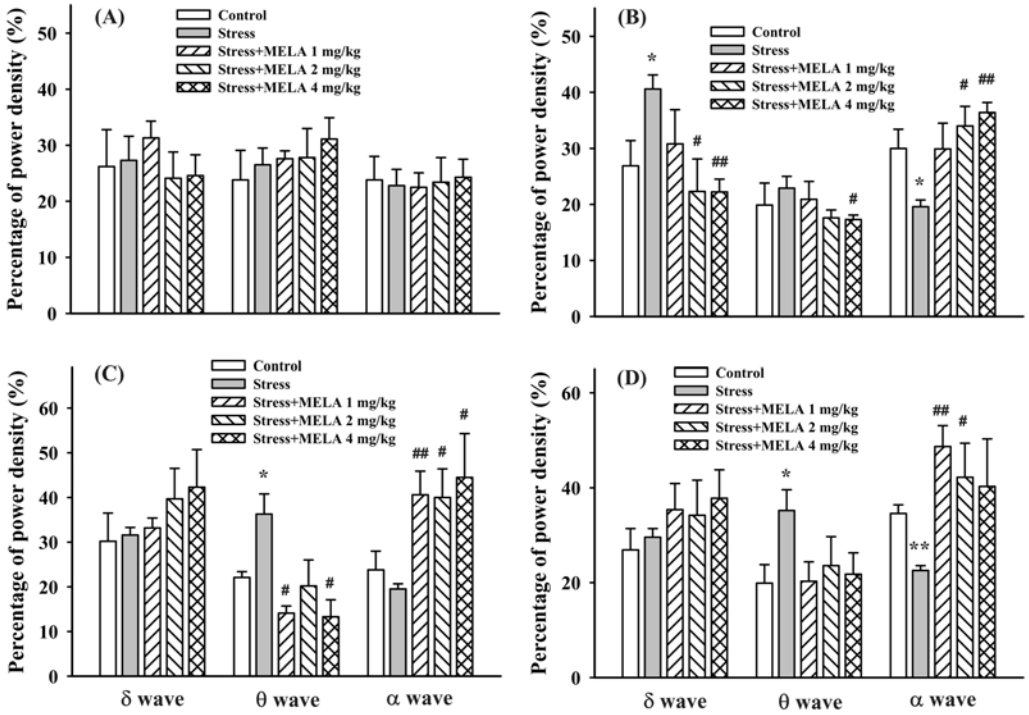

Fig. 4. Effects of MELA on EEG power density during REM sleep. The data represent mean ± SE of EEG power density in δ-wave, θ-wave, and α-wave bands during REM sleep. (A): Day time EEG power density during REM sleep in acutely stressed rats. (B): Night time EEG power density during REM sleep in acutely stressed rats. (C): Day time EEG power density during REM sleep in chronically stressed rats. (D): Night time EEG power density during REM sleep in chronically stressed rats. *P < 0.05; **P < 0.01, significantly different from control group; #P < 0.05; ##P < 0.01, significantly different from stress group.

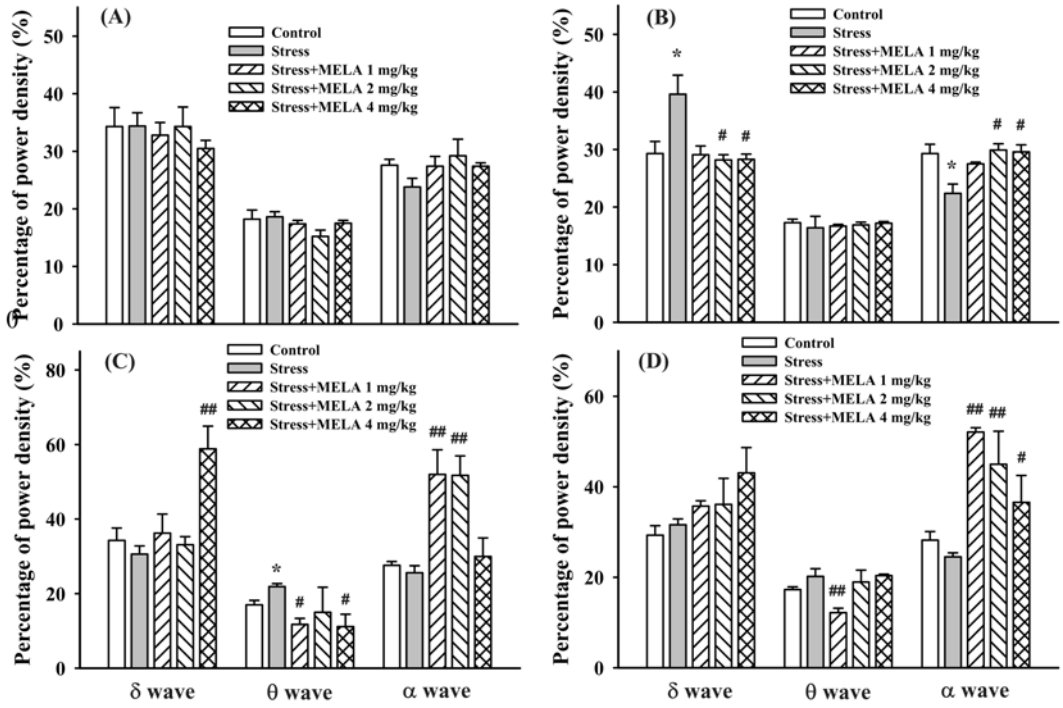

Fig. 5. Effects of MELA on EEG power density during wakefulness. The data represent mean ± SE of EEG power density in δ-wave, θ-wave, and α-wave bands during wakefulness. (A): Day time EEG power density during wakefulness in acutely stressed rats. (B): Night time EEG power density during wakefulness in acutely stressed rats. (C): Day time EEG power density during wakefulness in chronically stressed rats. (D): Night time EEG power density during wakefulness in chronically stressed rats. *P < 0.05, significantly different from control group; $^{\#}$P < 0.05; $^{\#\#}$P < 0.01, significantly different from stress group.

density of wakefulness. Acute stress change δ-wave and α-wave power density at night (δ-wave [F(4,35) = 3.21, $p < 0.05$]; α-wave [F(4,35) = 3.15, $p < 0.05$]). Chronic stress changed δ-wave, θ-wave and α-wave power density during the day (δ-wave [F(4,35) = 3.95, $p < 0.01$]; θ-wave [F(4,35) = 3.15, $p < 0.05$]; α-wave [F(4,35) = 4.13, $p < 0.01$]) and θ-wave and α-wave power density during the night (θ-wave [F(4,35) = 3.78, $p < 0.01$]; α-wave [F(4,35) = 4.41, $p < 0.01$]). During wakefulness, acute stress increased the night time δ-wave and decreased α-wave power density ($p < 0.01$, Fig. 5B), while chronic stress increased the day time θ-wave power density ($p < 0.05$, Fig. 5C). MELA at 2 and 4 mg/kg reduced night time δ-wave and increased α-wave power density in acutely stressed rats ($p < 0.05$, Fig. 5B). MELA decreased θ-wave and increased α-wave power density during the day and night after chronic stress, and 4 mg/kg MELA could also increase δ-wave power density in the day time EEG power density of wakefulness.

The regulation of the total amount of sleep is a homeostatic process (Datta and Mclean, 2007). Control mechanisms are activated to compensate for insufficient or excess sleep. In mammals, sleep consists of two major stages, NREM sleep and REM sleep. REM sleep is a distinct sleep stage that alternates with episodes of NREM sleep; the spontaneous NREM–REM sleep cycle in the rat takes about 12 to 20 min (Gottesmann, 1996; Datta and Hobson; 2000). Over the last four decades, most of the sleep research has focused on identifying relevant brain structures, neuronal networks, and the transmitters involved in the generation and regulation of NREM and REM sleep (Pace-Schott and Hobson, 2002; Blanco-Centurion *et al*., 2006). Few studies have focused on understanding the possible mechanisms and modulating methods for the ultradian periodic occurrence of NREM-REM sleep.

Normally, stress-induced changes are self-limiting and adaptive unless events that override the "threshold" limits become irreversible and pathological (Schurr, 2002). There are different responses in sleep behaviors after acute and chronic stress (Gilad and Gilad, 2002; Hayashi *et al*., 2004). The acute and chronic stress models of restraint stress in rats are well established. In this study, acute stress decreased wakefulness and increased total and REM sleep, especially at night. In contrast, total and NREM sleepings were significantly reduced. Chronic stress increased REM, particularly during the day. Cortical EEG activity also showed significant differences

in acute and chronic stress. These results agree with previous reports and suggest that cortical EEG activity could reflect stress conditions (Hayashi *et al*., 2004; Sohn *et al*., 2002).

Longanae Arillus is used extensively to improve sleep quality and mental wellbeing as it possesses sleep stabilizing effects. MELA increased wakefulness in acutely stressed rats and decreased total and REM sleep, particularly at night. MELA increased total, NREM sleep, and REM sleep, and decreased wakefulness in chronic stress, but did not change the number of sleep-wake cycles. These results are consistent with the belief that Longanae Arillus is effective in ameliorating stress-related sleep disorders as they relate to different patterns induced by acute or chronic stress (Ma *et al*., 2009a).

The modulation of total amount and the frequency of sleep depend on the specific activity patterns of cortical EEG waves (Franken *et al*., 2001). NREM sleep in experimental animals, also referred to as slow wave sleep (SWS), is a deep sleep stage in humans and other mammals; it is defined by an oscillation of the EEG in the δ-wave frequency range (< 2 Hz in human, 0.75 to 4.0 Hz range in rats) (Maret *et al*., 2005). EEG activities in the δ-wave frequency delineate NREM sleep and represent widespread, synchronized firing patterns of cortical and thalamocortical neurons that modulate sleep (Steriade *et al*., 1993). Sleep deprivation, or forced wakefulness, can induce a large increase in the δ-wave power. This mechanism of sleep is essential for rest and maintenance of neural function. The regulation of the δ-wave activity in sleep is linked to synaptic potentiation and downscaling, with increases in the local δ-wave activity in sleep after a motor learning task correlating with improved performance in humans (Kitaoka *et al*., 2007). The power of the δ-wave activity is a reliable parameter for an assessment of sleep depth and the regulation of sleep (Borbely, 2001). δ-Wave activity relates to prior waking and is also a reliable indicator of time spent awake. The intensity of δ-wave activity in the cortical EEG is the single most important process for the regulation of NREM sleep. The ä-wave activity in NREM sleep typically declines in the course of the daily sleep period and increases in recovery sleep after a period of prolonged waking (Franken *et al*., 2001). Furthermore, δ-wave activity is reduced in the subsequent NREM sleep after a nap and/or following a period of excess sleep (Frinberg *et al*., 1992). However, MELA decreased the NREM and REM sleep with decreased δ-wave activity. In particular, MELA decreased the night time REM sleep of acutely stressed rats. Therefore, MELA may modulate the sleep architecture in a similar fashion to the physiological regulation that occurs during a nap or a period of excess sleep in acute stress. In chronic stress, a high dose of MELA (100 mg/kg) increased NREM and decreased REM sleep and was accompanied by an increase of δ-wave activity during nighttime NREM sleep and daytime REM sleep. This indicates that δ-wave activity is closely related to chronic stress induced sleep alterations and sleep modulation by MELA.

Unlike NREM sleep, the EEG correlation for the REM sleep process remains poorly understood. Some studies in humans have suggested that the EEG activity of the α-wave activity (frequency range of 8-13 Hz) might be a marker of REM sleep regulation. However, another study in the rat has suggested that the α-wave activity may not be involved in REM sleep. Thus, δ-wave activity remains the only parameter conclusively involved in the regulation of REM sleep (Roth *et al*., 1999; Bjorvatn *et al*., 1998; Shea *et al*., 2008).

In this study, 2 and 4 mg/kg of MELA decreased total and REM sleep and increased wakefulness at night in acutely stressed rats, decreasing δ-wave and increasing α-wave activity in night time REM sleep and wakefulness. MELA did not change the sleep architecture and EEG power density of δ-, θ- and α-wave activity on NREM or REM sleep and wakefulness during the day. In chronically stressed rats, MELA increased total sleep and decreased wakefulness during the day and night and increased NREM and REM sleep strongly during the day; MELA increased NREM sleep δ-wave as well as REM sleep and wakefulness α-wave power density both day and night, but MELA decreased the θ-wave activity of NREM and REM sleep and wakefulness during the day. These differences indicate that MELA is more effective in modulating acute stress-induced sleep disorders at night and chronic stress-induced sleep disorders during the day. These results also suggest that the effects of MELA are more specifically related to the function of REM and NREM sleep. MELA may affect GABAergic systems by interacting with ligand binding to $GABA_A$ or $GABA_B$ receptors. MELA in animals induces behavioral changes related to the regulation of GABAergic neurotransmission (Ma *et al*., 2009a). Insomnia is one of the most frequent complaints encountered clinically and is most often related to anxiety and/or stress (Gottesman, 1996) GABAergic modulation by MELA is possibly involved in the alternation of sleep architecture and EEG power spectra in stressed animals. Further study is necessary to clarify the possible mechanisms.

In conclusion, we demonstrated that acute and chronic

stresses induce sleep disorders with different patterns. MELA increased wakefulness and decreased the total and REM sleep in acutely stressed rats; MELA also increased NREM and REM sleep in chronically stressed rats. Regulation of the sleep architecture of MELA is focused on REM and NREM sleep, and involves modulation of α-, θ- and δ-wave activities in the cortical EEG. Novel, active compounds from MELA could modulate sleep through specific mechanisms and should be the subject of further studies on sleep architecture and EEG wave activity.

Acknowledgements

This work was supported by a Grant from Nonghyup Korea Ginseng and the Korean Ministry of Education, Science and Technology (The regional Core Research Program/Center for Healthcare Technology Development).

References

Bjorvatn, B., Fagerland, S., and Ursin, R., EEG power densities (0.5-20 Hz) in different sleep-wake stages in rats. *Physiol. Behav.*, **63**, 413-417 (1998).

Blanco-Centurion, C., Xu, M., Murillo-Rodriguez, E., Gerashchenko, D., Shiromani, A.M., Salin-Pascual, R.J., Hof, P.R., and Shiromani, P.J., Adenosine and sleep homeostasis in the basal forebrain. *J. Neurosci.*, **26**, 8092-8100 (2006).

Bodosi, B., Obal, F. Jr, Gardi, J., Komlodi, J., Fang, J., and Krueger, J. M., An ether stressor increases REM sleep in rats: possible role of prolactin. *Am. J. Physiol.*, **279**, R1590-1598 (2000).

Bonnet, C., Marinesco, S., Debilly, G., Kovalzon, V., and Cespuglio, R., Influence of a 1-h immobilization stress on sleep and CLIP (ACTH(18-39)) brain contents in adrenalectomized rats. *Brain Res.*, **853**, 323-329 (2000).

Borbely, A.A., From slow waves to sleep homeostasis: new perspectives. *Arch. Ital. Biol.*, **139**, 53-61 (2001).

Datta, S. and Hobson, J.A., The rat as an experimental model for sleep neurophysiology. *Behav. Neurosci.*, **114**, 1239-1244 (2000).

Datta, S. and Maclean, R.R., Neurobiological mechanisms for the regulation of mammalian sleep-wake behavior: reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence. *Neurosci. Biobehav. Rev.*, **31**, 775-824 (2007).

Feinberg, I., Maloney, T., and March, J.D., Precise conservation of NREM period 1 (NREMP1) delta across naps and nocturnal sleep: implications for REM latency and NREM/REM alternation, *Sleep* **15**, 400-403 (1992).

Festa, F., Aglitti, T., Duranti, G., Ricordy, R., Perticone, P., and Cozzi, R., Strong antioxidant activity of ellagic acid in mammalian cells in vitro revealed by the comet assay. *Anticancer Res.*, **21**, 3903-3908 (2001).

Franken, P., Chollet, D., and Tafti, M., The homeostatic regulation of sleep need is under genetic control. *J. Neurosci.*, **21**, 2610-2621 (2001).

Gilad, G.M. and Gilad, V.H., Stress-induced dynamic changes in mouse brain polyamines: Role in behavioral reactivity. *Brain Res.*, **943**, 23-29 (2002).

Gonzalez, M.M., Debilly, G., Valatx, J.L., and Jouvet, M., Sleep increase after immobilization stress: role of the noradrenergic locus coeruleus system in the rat. *Neurosci. Lett.*, **202**, 5-8 (1995).

Gottesmann, C., The transition from slow-wave sleep to paradoxical sleep: evolving facts and concepts of the neurophysiological processes underlying the intermediate stage of sleep. *Neurosci. Biobehav. Rev.*, **20**, 367-387 (1996).

Hayashi, Y., Tanaka, J., Morizumi, Y., Kitamura, Y. and Hattori, Y., Polyamine levels in brain and plasma after acute restraint or water-immersion restraint stress in mice. *Neurosci. Lett.*, **355**, 57-60 (2004).

Kant, G.J., Pastel, R.H., Bauman, R.A., Meininger, G.R., Maughan, K.R, Robinson, T.N 3rd., Wright, W.L. and Covington, P.S., Effects of chronic stress on sleep in rats. *Physio. Behav.*, **57**, 359-365 (1995).

Kawada, M., Ohno, Y., Ri, Y., Ikoma, T., Yuugetu, H., Asai, T., Watanabe, M., Yasuda, N., Akao, S., Takemura, G., Minatoguchi, S., Gotoh, K., Fujiwara, H. and Fukuda, K., Anti-tumor effect of gallic acid on LL-2 lung cancer cells transplanted in mice. *Anticancer Drugs*, **12**, 847-852 (2001).

Kitaoka, K., Hattori, A., Chikahisa, S., Miyamoto, K., Nakaya, Y., and Sei, H., Vitamin A deficiency induces a decrease in EEG delta power during sleep in mice. *Brain Res.*, **1150**, 121-130 (2007).

Ma, Y., Kim, Y.B., Nam, S.Y., Cheong, J.H., Park, S.H., Kim, H.J., Hong, J.T., and Oh, K.W., Effects of red ginseng extract on sleep architecture and electrpencephalogram power spectra in rats. *Sleep Biol. Rhythms*, **7**, 78-83 (2009).

Ma, Y., Ma, H., Eun, J.S., Nam, S.Y., Kim, Y.B., Hong, J.T., Lee, M.K., and Oh, K.W., Methanol extract of Longanae Arillus augments pentobarbital-induced sleep behaviors through the modification of GABAergic systems. *J. Ethnopharmacol.*, **122**, 245-250 (2009).

Maret, S., Franken, P., Dauvilliers, Y., Ghyselinck, N.B., Chambon. P., and Tafti, M., Retinoic acid signaling affects cortical synchrony during sleep. *Science*, **310**, 111-113 (2005).

Okuyama, E., Ebihara, H., Takeuchi, H., and Yamazaki, M., Adenosine, the anxiolytic-like principle of the Arillus of *Euphoria longana*. *Planta Med.*, **65,** 115-119 (1999).

Pace-Schott, E.F. and Hobson, J.A., The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nature Rev.*, **3**, 591-605 (2002).

Rampin, C., Cespuglio, R., Chastrette, N., and Jouvet, M., Immobilization stress induces a paradoxical sleep rebound in rat. *Neurosci. Lett.*, **126**, 113-118 (1991).

Rangkadilok, N., Sitthimonchai, S., Worasuttayangkurn, L., Mahidol, C., Ruchirawat, M., and Satayavivad, J., Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. *Food Chem. Toxicol.*, **45**, 328-336 (2007).

Rangkadilok, N., Worasuttayangkurn, L., Bennett, R.N., and Satayavivad, J., Identification and quantification of polyphenolic compounds in Longan (*Euphoria longana* Lam.) fruit. *J. Agric. Food Chem.*, **53**, 1387-1392 (2005).

Roth, C., Achermann, P., and Borbely, A.A., Alpha activity in the human REM sleep EEG: topography and effect of REM sleep deprivation. *Clin. Neurophysiol.*, **110,** 32-635 (1999).

Sanford, L.D., Yang, L., Liu, X., and Tang, X., Effects of tetrodotoxin (TTX) inactivation of the central nucleus of the amygdala (CNA) on dark period sleep and activity. *Brain Res.*, **1084**, 80-88 (2006).

Schurr, A., Energy metabolism, stress hormones and neural recovery from cerebral ischemia/hypoxia. *Neurochem. Internat.*, **41**, 1-8 (2002).

Shea, J.L., Mochizuki, T., Sagvaag, V., Aspevik, T., Bjorkum, A.A., and Datta, S., Rapid eye movement (REM) sleep homeostatic regulatory processes in the rat: changes in the sleep-wake stages and electroencephalographic power spectra. *Brain Res.*, **1213**, 48-56 (2008).

Sohn, H.S., Park, Y.N., and Lee, S.R., Effect of immobilization stress on brain polyamine levels in spontaneously hypertensive and Wistar-Kyoto rats. *Brain Res. Bull.*, **57**, 575-579 (2002).

Steriade, M., McCormick, D.A., and Sejnowski, T.J., Thalamocortical oscillations in the sleeping and aroused brain. *Science*, **262**, 679-685 (1993).

Sun, J., Shi, J., Jiang, Y., Xue, S.J., and Wei, X., Identification of two polyphenolic compounds with antioxidant activities in longan pericarp tissues. *J. Agric. Food Chem.*, **55**, 5864-5868 (2007).

Vazquez-Palacios, G., Retana-Marquez, S., Bonilla-Jaime, H., and Velazquez-Moctezuma, J., Stress-induced REM sleep increase is antagonized by naltrexone in rats. *Psychopharmacol.*, **171**, 186-190 (2004).

Yilmaz, Y. and Toledo, R.T., Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin, and gallic acid. *J. Agric. Food Chem*, **52**, 255-260 (2004).

Received November 9, 2009
Revised December 9, 2009
Accepted December 9, 2009