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Sleep-Aids Derived from Natural Products

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Abstract

Although drugs such as barbiturates and benzodiazepines are often used for the treatment of insomnia, they are associated with various side effects such as habituations, tolerance and addiction. Alternatively, natural products with minimal unwanted effects have been preferred for the treatment of acute and/or mild insomnia, with additional benefits of overall health-promotion. Basic and clinical researches on the mechanisms of action of natural products have been carried out so far in insomnia treatments. Recent studies have been focusing on diverse chemical components available in natural products, with an interest of developing drugs that can improve sleep duration and quality. In the last 15 years, our co-workers have been actively looking for candidate substances from natural products that can relieve insomnia. This review is, therefore, intended to bring pharmacological data regarding to the effects of natural products on sleep duration and quality, mainly through the activation of $GABA_A$ receptors. It is imperative that phytochemicals will provide useful information during electroencephalography (EEG) analysis and serve as an alternative medications for insomnia patients who are reluctant to use conventional drugs.

Key Words: Insomnia, Sleep, Natural products, Pharmacological mechanisms, Electroencephalography (EEG)

AN OVERVIEW OF INSOMNIA

Most people often or chronically experience insomnia characterized by difficulty in falling asleep, overnight loss of sleep, trouble to resume sleep, waking up too early, unable to refreshed after sleep and loss of working time due to tiredness and faintness (National Sleep Foundation, USA). Sleep is vital to maintain mood, memory, cognitive function by restoring the majority of human body systems through endocrine and immune functions. Therefore, sleep is one of the most instinctive and essential physiological demand for normal life such as maintaining health and mental stability. Meanwhile, humans may suffer from various sleep disorders, including insomnia, hypersomnia, narcolepsy, and sleep apnea (Jacobson *et al*., 2017).

On the other hand, insomnia has been associated with psychiatric conditions, unhealthy sleep habits, specific excitatory substances, and/or certain biological factors. Moreover, medical conditions with comorbidities may aggravate these insomnia related abnormalities (Starks *et al*., 2018).

So far, studies have been focusing on various molecules leading into insomnia. Many of these molecules involved in sleep-wake regulation are produced by specific brain regions with widespread projections. Research findings have been largely interpreted within the context of hyperarousal hypothesis. For instance, insomnia patients with GABA in the occipital cortex has been reported to be consistent with the hyperarousal model of insomnia. In addition, emotional and cognitive systems lead to suppression of sleep-promoting regions such as the ventrolateral preoptic area (VLPO) (Wang and Liu, 2016; Bourcier *et al*., 2018). In addition, orexin/hypocretin neurons of the lateral hypothalamus project to all of the sleep/insomnia arousal-promoting centers in the brainstem and hypothalamus thereby reinforcing their activity to play on important roles in sleep. The pathophysiological understanding of insomnia may provide important information regarding how, and under what conditions, the disorder develops and is maintained as well as potential targets for prevention and treatment (Jacobson *et al*., 2017).

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ANIMAL SURGERY & EEG RECORDING

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and the transmitter was implanted for recording EEG via telemetry as described earlier (Sanford *et al*., 2006). Briefly, the body of the transmitter was subcutaneously implanted just posterior to the scapula with three sutures for stabilization. The transmitters led subcutaneously to the skull, and the bare ends were placed in contact with the dura through holes in the skull. The electrodes were anchored to the skull with screws and dental cement. All of the surgical procedures were performed stereotaxically under aseptic conditions (Yang *et al*., 2011b; Perentos *et al*., 2017).

For telemetric recording of signals from cortical EEG, the gain of transmitters was set at –0.5/+0.5 volts per units ×2 with raw signals ranging from 0.5 to 0.0 Hz and these signals were processed by Data Sciences analog converter and routed to an analog-to-digital (AD) converter (Eagle PC30, Data Sciences International). The AD converter digitized the EEG and activity signals; subsequently data were transferred to a computer and graphically displayed. An on-line fast Fourier transformation (FFT) analyzed EEG data and generated power density values from 0.0 to 20.0 Hz at a resolution of 0.5 Hz. The FFT data were further averaged between 0 to 20 Hz at 10-s intervals. The sleep data and FFT results were saved to the hard disk every 10 s for additional off-line analysis. Movements of the animal in relation to the telemetry receiver generated transistor-transistor logic pulses that were collected and counted as a measure of activity. The signal of EEG was measured for 6 hours between 11:00 am and 5:00 pm. Each group has 5-6 rats (Hu *et al*., 2013).

Time elapsed in wakefulness, NREM sleep or REM sleep was determined from digitized data within 10 s using the animal sleep analysis software Sleep-Sign 2.1 (Kissei Comtec, Matsumoto, Japan). Briefly, the software identifies wakefulness as a high-frequency with low-amplitude of EEG whereas in NREM sleep, it showed spindles interspersed with slow waves compared with REM sleep characterized by δ-waves (0.75 to 4.0 Hz) and θ-wave activity (5.0 to 9.0 Hz) with peak value at 7.5 Hz.

MOLECULAR TARGETS FOR INSOMNIA THERAPY

Several efficacious pharmacological treatments for insomnia target to various aspects of identified pathophysiologic processes. For instance, GABA is released from the terminal of specific inhibitory neurons and then it binds to its receptors, thereby enhancing chloride influx and facilitates $GABA_A$-ergic transmission (Olsen, 1981; Ticku and Maksay, 1983). This effect is supported by benzodiazepines and barbiturates by their agonistic effects on the $GABA_A$ receptors. Thus, their binding to the allosteric site of the receptors enhances the affinity of the GABA-binding site. However, four different types of $GABA_A$ receptor subunits have been described, each of which encloses different membranes. Likewise, the critical step in GABA biosynthesis is the decarboxylation of glutamate by glutamic acid decarboxylase (GAD), which exists in two different isoforms, GAD_{65} and GAD_{67}. The level of GAD_{65} and GAD_{67} is reported to be up-regulated in the $GABA_A$-ergic interneurons. With this intricate nature, $GABA_A$ receptors have been known to play an important role in the modulation of barbiturate-induced sleeping through interaction with $GABA_A$-ergic systems (Doghramji, 2006). GABA-benzodiazepine receptor agonists which are generally effective in the treatment of insomnia can promote sleep by enhancing the widespread function of GABA. This suggest that new compounds can be developed for specific molecular targets with known sleep-related actions. Apart from this, non-$GABA_A$-chloride channel receptor complex agonist such as melatonin (MT) receptor agonist, 5-HT_{1A} receptor agonist, orexin receptor agonist, adenosine receptor agonist and histamine receptor antagonist have been suggested for the treatment of insomnia (Dujardin *et al*., 2018). Benzodiazepines are currently the most well-known and most frequently prescribed hypnotic medications, although their use in recent years is being replaced by newer non-benzodiazepine hypnotic drugs, so-called “z-drugs” such as eszopiclone, zolpidem and zaleplon. As these non-benzodiazepine medications are generally believed to be better and safer than earlier generations of sedatives, they have still generated some controversy and discussion regarding side-effects (Schwartz *et al*., 2017).

The possible role of serotonin (5-HT) in human sleep disorders has been considered. Ursin provides an excellent overview of the evolving concept of the role of serotonin (5-HT) in sleep, supporting the sleep-promoting effects (Ursin, 2002). The role of specific receptor subtypes in sleep-wake regulation has been focused. Gronli reported the effect of certain drugs affecting 5-HT_{1A} and 5-HT_{1B} receptors, which have affinity at the pre- and post-synaptic receptors, later including inhibitory auto-receptors on serotonin neurons, themselves (Grønli *et al*., 2007). In particular, 5-$HT_{1A/1B}$, plays a crucial role in regulating serotonin transmission in the brain. Recent studies have also suggested a role for the 5-HT_{1B} receptor in depression, anxiety and sleep. 5-HT_{1B} antagonists reduce the latency to onset of anxiolytic behavior and play a role in stress regulation with activity comparable to diazepam (Tatarczynska *et al*., 2004). However, some antagonists of serotonin may increase non-rapid eye movement (NREM) sleep. The findings are complex, but support the role of 5-HT_{1A}, 5-HT_{1B} and 5-HT_7 receptors in REM control (Leiser *et al*., 2015).

Non-benzodiazepine hypnotics such as diphenhydramine and doxylamine have been used for the treatment of insomnia. The sedative effect of antihistamines is mainly achieved by targeting the histamine receptors in arousal systems. More recently, there has been progress in the development of orexin receptor antagonists for the treatment of insomnia. Orexin systems target on the promotion of arousal of brainstem/hypothalamic arousal centers (Mieda, 2017). In addition to this, other molecular targets such as melatonin and adenosine have been suggested for the treatment of insomnia (Di Bella *et al*., 2017).

Various compounds, with novel approaches are being evaluated currently as possible insomnia treatments. Currently, Korea FDA is reviewing new applications for innovative sleep-promoting herbs. Clinical indications have been developed for insomnia associated with problem of sleep onset, sleep maintenance, and middle-of-the-night awakenings. Alternative approaches to treating insomnia have included an off-label basis for insomnia, over-the-counter sleep aids, and assorted unregulated substances marketed to enhance sleep. Substances regarded as appropriate hypnotics are those which prevent continuous awakenings, shorten the period of latency for sleep initiation and increase sleep duration, besides displaying low toxicity.

Fig. 1. EEG measurement experiment in the rats. 1. To measure EEG changes, the rats were sutured after implantation of the transmitter body on the back of male rats. 2. The electrode is inserted and fixed with dental cement after puncturing the skull. 3. After one week of recovery from surgery, EEG is recorded. 4. The sleep architecture is analyzed based on the EEG record, which is received on the computer.

Key physiological measurements indicators of sleep include electroencephalography (EEG) of brain waves, electrooculography (EOG) of eye movements, electromyography (EMG) of skeletal muscle activity. Simultaneous collection of these measurements is called polysomnography, and can be performed in a specialized sleep laboratory (Rundell and Jones, 1990). Sleep researchers also use simplified electrocardiography (ECG) for cardiac activity and actigraphy for motor movements (Jafari and Mohsenin, 2010). This has promoted the search for alternative approaches such as the employment of phythotherapeutic agents. Animal behavioral experiments were done after surgery and agent treatment (Fig. 1).

NATURAL PRODUCTS WHICH HAVE PROVEN TO BE EFFECTIVE FOR SLEEPING FROM OUR LABORATORY

A number of medicinal plants are traditionally endowed with anxiolytic or sedative properties. This has motivated the searching of phythotherapeutic agents, which has been used in animal behavioral experiments performed by surgery (Fig. 1). In this context, there is a lot of information in medicinal plants possessing sedative and hypnotic properties. Here, we describe some natural products studied in our laboratory. These are magnolia (*Magnolia officinalis*, Magnoliaceae), Semen zizyphi spinosae (*Zizyphus jujube* Mill. var. *spinosa* Rhamnaceae), sinomenine (*Sinomenium acutum* Menispermaceae), decursinol (*Angelica gigas*, Unbelliferae), rosemary (*Perilla frutescens*, Lamiaceae), *Euphoria longan* (Spindaceae), Ginseng (*Panax ginseng*, Araliacae), EGCG (Epigallocatechin-3-O-gallate, *Camellia sinensis*), *Chrysanthemum morifolium* (Asteraceae), Apigenin (*Circium japonicum*, Asteraceae) and some others that possess sedative effects (Fig. 2).

Magnolia (*M. obovate*, *M. officinalis*, Magnoliaceae) has been traditionally used for the treatment of thrombotic stroke, depression, anxiety, and inflammatory and neuronal diseases in oriental countries for a long time (Watanabe *et al*., 1983; Hirano, 1991; Lo *et al*., 1994). Biophenolic compounds such as magnolol, honokiol, and obovatol isolated from *M. obovate*/ and *M. officinalis* have been found to be anxiolytic and muscle relaxant (Maruyama *et al*., 1998; Seo *et al*., 2007; Han *et al*., 2010). From a previous experiment, it was reported that obovatol has anxiolytic-like effects in animal models, and enhanced pentobarbital-induced sleep suggesting that these effects are involved in GABA/benzodiazepine receptor complex (Seo *et al*., 2007; Ma *et al*., 2009b). In addition, research has shown that magnolol and honokiol are the primary active components of *M. officinalis* and they are positive allosteric modulators of $GABA_A$ receptors. In addition, they have shown to increase the density of $GABA_A$ receptors that possess alpha subunits and this has been a mechanism followed by diazepam, a benzodiazepine sometimes used to treat insomnia, (Ma *et al*., 2008, 2009b; Lee *et al*., 2010). All together, these effects increase GABA activity, which promotes relaxation and reduces anxiety. Magnolol increased the amount of REM and NREM sleep via the $GABA_A$ receptors (Chen *et al*., 2012). Particularly, magnolia bark has been used for human treatment as an anxiolytic, helping to lower anxiety, depression, reduce stress, and facilitating sleep. These effects could be indicators of its positive potential as a natural sleep aiding agent.

Semen zizyphi spinosae (Rhamnaceae, the dried seed of *Zizyphus jujube* Mill *var. spinosa*) has been used as a tranquilizer, an anxiolytic and anticonvulsant in oriental countries, and also has been prescribed for the treatment of insomnia and anxiety (Park *et al*., 2004). It was reported that sanjoinine, which is a major alkaloid compound, and cyclopeptides from Semen zizyphi spinosae have have shown anxiolytic-like effects in the elevated plus-maze, hole-board test and open field test, and these effects may be mediated by $GABA_A$-ergic transmission (Han *et al*., 2008). Sanjoinine A and cyclopeptide fraction from Semen zizyphi spinosae exert hypnotic effect and/or enhances pentobarbital-induced sleeping behaviors (Ma *et al*., 2008; Han *et al*., 2009). Sanjoinine A in combination with muscimol showed synergistic effects on pentobarbital-induced sleeping and increased Cl^- influx in a similar way as pentobarbital. Sanjoinine A also showed similar effects with muscimolin potentiating Cl^- influx inducing the effects of low dose pentobarbital. This indicated that sanjoinine A might act on $GABA_A$ receptor to induce Cl^- channel opening, and modulate pentobarbital-induced pharmacological properties like a $GABA_A$ receptor agonist (Ma *et al*., 2007). Moreover, cyclo-

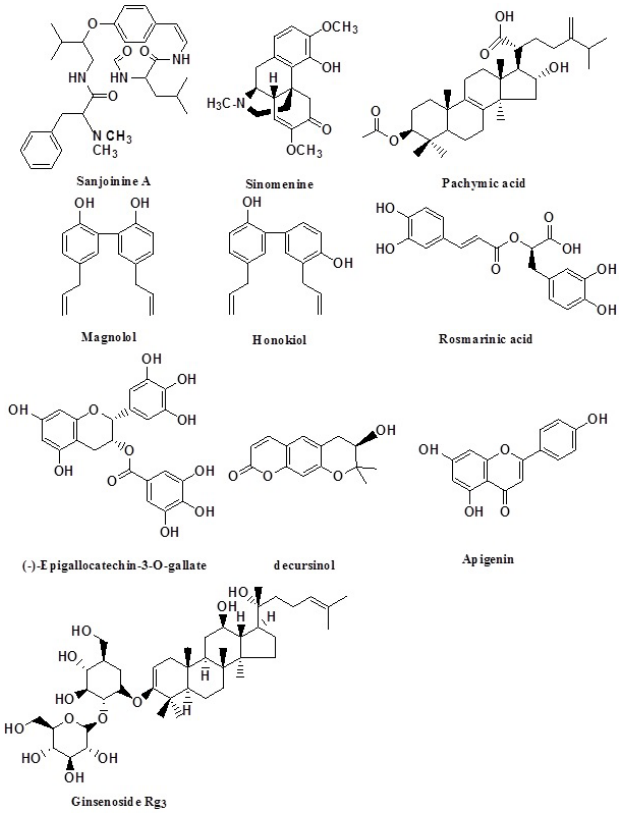

Fig. 2. Chemical structures originated from natural products which have been to be effective for sleep from our laboratory.

peptide alkaloid fraction of semen *zizyphi spinosae* increased pentobarbital-induced sleeping behaviors, through activation of GABA receptor Cl^- channels (Ma *et al*., 2008). However, it has been suggested for high affinity for 5-HT_1a and 5-HT_2 receptors in *in vivo* analysis (Yi *et al*., 2007).

Sinomenine, an alkaloid derived from *Sinomenium acutum*, is a chief ingredient that has been reported to have a variety of pharmacological properties including anxiolytic effects (Rao *et al*., 2017). From a recent experiment, sinomenine enhanced pentobarbital-induced sleeping behaviors, and modulate sleep architecture via $GABA_A$-ergic systems in rodents, ultimately increasing NREM sleep.

The most abundant $GABA_A$ receptor subunit compositions, α1, β2 and γ2, are related to the hypnotic/sedative effect of $GABA_A$ receptors (Rudolph and Möhler, 2006). Previous studies have shown that α1 subunit was associated with sedation (Rudolph and Feiger, 1999; McKernan *et al*., 2000). Whereas α2/3 subunits were associated with anxiety (Löw *et al*., 2000; Crestani *et al*., 2001), α5 subunit was associated with temporal and spatial memory (Collinson *et al*., 2002; Crestani *et al*., 2002). Sinomenine non-selectively activated these subunits of $GABA_A$ receptors. During our experiment, major subunits of $GABA_A$ receptor were over-expressed by sinomenine except for α3 and α5 subunits

Decursinol is one of the major components of *Angelica gigas* (Umbelliferea) which has been used for a long time as a traditional folk medicine in oriental countries. This herb has been used traditionally for the treatment of psychosomatic disease such as excess stress, anxiety, depression and insomnia. It has been reported that the essential oil components of *Angelica gigas* exhibited anxiolytic-like effects in rodent tests (Chen *et al*., 2004). Similarly, Japanese Angelica root extract reversed stress-induced loss of sleep in pentobarbital-induced sleeping in mice through the inhibition of the central noradrenergic or the activation of $GABA_A$ receptors (Matsumoto *et al*., 1998). Decurcinol increased the number of sleeping animals in the sub-hypnotic dosage and modulated sleep architectures, most likely by increasing the protein expression of glutamic acid decarboxylase ($GAD_{65/67}$) and $GABA_A$ receptors subtypes. On the other hand, decurcinol potentiates pentobarbital-induced sleeping behaviors through the activation of $GABA_A$-ergic systems. Altogether suggest that decurcinol, can be useful agent for the treatment of insomnia (Woo *et al*., 2017).

Rosemary (*fromPerilla frutescens, Lamiaceae*) has been used as a folk remedy for sedation in oriental countries. So far, several studies have shown that *Perilla frutescens* has sedative effect (Takeda *et al*., 2002; Johnston *et al*., 2006). Rosemary contains a number of phytochemicals, including rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol (Vallverdú-Queralt *et al*., 2014). Rosmarinic acid belonging to the phenolic compounds is a constituent of *Perilla frutescens* (Igarashi and Miyazaki, 2013; Rašković *et al*., 2014). Many of the phenolic compounds originating from plants have been known to affect the GABA-ergic systems (Johnston *et al*., 2006). In addition, rosmarinic acid inhibited GABA transaminase (GABA-T) *in vitro* (Awad *et al*., 2009), and increased the protein expression of $GAD_{65/67}$ and $GABA_A$ receptors subunits except β1 subunit. Rosmarinic acid augmented pentobarbital-induced sleeping behaviors through $GABA_A$-ergic transmission. It is suggested that rosmarinic acid may be useful agent for the treatment of insomnia (Kwon *et al*., 2017).

Euphoria longan (*or Dimocarpus longan*, Spindaceae) is commonly consumed as the dried fruit of Euphoria longana. Phytochemicals such as gallic acid, ellastic acid and corilagin (ellagitanin) were identified from the peel, seed and pulp of its fruit (Rang-Kadilok). The pulp of the dried fruit and fresh Longanae Arillus, has been used for the treatment of anxiety and insomnia in Asian countries. The extract of Longane Arillus was proven to have anxiolytic activity (Okuyama *et al*., 1999). From our experiment, methanol extract of Longane Arillus was proven to prolong sleeping time and reduced pentobarbital-induced sleep onset in rodents through $GABA_A$-ergic systems (Ma *et al*., 2009b). Methanol extract of Longane Arillus regulated sleep architectures and EEG power spectra in restraint-stressed rats (Ma *et al*., 2009b).

Ginseng (*Panax ginseng*, Araliaceae) may be in part related to maintaining sleep and wakefulness, and alter EEG spectra of sleep-wake stage in mice (Ma *et al*., 2008, 2009a, 2009b; Yang *et al*., 2011b). *Panax quinquefolium* (American ginseng) were reported to have sleeping-modulating effects. Saponin fraction of *Panax ginseng* extract prolonged the duration of hexobarbital-induced sleep in mice (Takagi *et al*., 1972). In addition, red ginseng extract increased total sleep time and NREM sleep (Ma *et al*., 2008; Yang *et al*., 2011a). Majonosides R2, a major saponin of *Panax vietnamensis* restored the hypnotic activity of pentobarbital, which was decreased by psychological stresses (Nguyen *et al*., 1993). Ginsenoside Rg3-standarzied ginseng extract showed anti-stress effects and enhanced sleeping in restraint stressed animals (Kim *et*

al., 2010). There are evidences to suggest that the regulation of $GABA_A$-ergic transmission is one of target for the sedative actions of ginseng (Kimura *et al*., 1994; Cha *et al*., 2005; Park *et al*., 2005). In human study, it has been confirmed that ginseng could improve the quality of sleep (Han *et al*., 2013). Korea red ginseng increased NREM sleep via $GABA_A$-ergic systems (Lee *et al*., 2012).

EGCG (Epigallocatechin-3-O-gallate) is a major component of green tea (*Camellia sinensis*) which is a popular beverage worldwide. However, green tea contains caffeine and EGCG. One is stimulant caffeine, and the other is EGCG which may be sedative. From our experiment, it was interesting that only EGCG from green tea augmented pentobarbital-induced sleeping time through Cl^- channel activation (Park *et al*., 2011). This may suggest that EGCG counteracts caffeine-induced stimulant effects such as hyperactivity, arousal and anxiogenic effects (Park *et al*., 2010). Anxiolytic effects and enhancement of sleep by EGCG also could be medicated by $GABA_A$-ergic systems (Bae *et al*., 2002; Campbell *et al*., 2004; Vignes *et al*., 2006; Haque *et al*., 2008; Park *et al*., 2011).

Chrysanthemum morifolium (Asteraceae). The flower *Chrysanthemum morifolium* is a medicinal herb that has been used for tea preparation in oriental countries. *Chrysanthemumsp* contains flavonoids, phenols, cinnamic acids and on. The dry flower of *Chrysanthemum morifolium* in tea and pillow has been traditionally used for the treatment of insomnia in Korea. Ethanol extract of *Chrysanthemum morifolium* augments pentobarbital-induced sleeping behaviors through activation of Cl^- channels (Kim *et al*., 2011).

Apigenin (from *Circium japonicum*, Asteraceae) found in many plants, is a natural product belonging to flavonoids that are the aglycone of several naturally occurring glycosides. Apigenin is a weak ligand for central benzodiazepine receptors *in vitro* and exerts anxiolytic and slight sedative effects in an animal model (Viola *et al*., 1995). Apigenin shows second-order positive modulatory activity at $GABA_A$ receptors (Campbell *et al*., 2004; Rosi *et al*., 2004). It was reported that enhancement of pentobarbital-induced sleep by apigenin is mediated by $GABA_A$ receptors through Cl^- channel complex activation (Kim *et al*., 2012).

Others. Some natural products such as Polygalae Radix (3,4,5-trimethoxycinnamic acid), Gastrodiae Rhizoma (4-hydroxybenzaldehyde), *Poria cocos* (pachymic acid), Rhynchophilline (*Uncariae Ramulus et Uncus*) and Perilla Herba, were studied whether they enhance sleep or not (Lee *et al*., 2013; Choi *et al*., 2014; Shah *et al*., 2014; Choi *et al*., 2015; Shah *et al*., 2015; Kwon *et al*., 2017). From the behavioral and molecular data, those natural products augmented pentobarbital-induced sleep, and modulated sleep architectures of EEG spectra in rodents. The increase of sleep seems to be mediated through $GABA_A$-benzodiazepine receptors Cl^- channel complex.

However, cordycepin (*Cordyceps militaris/sinensis*) increased theta waves power density during NREM sleep. Adenosine receptors (AR) subtypes (A1, A2A and A2B) were overexpressed by cordycepin, and they are showing non-specific AR activation (Hu *et al*., 2013).

CONCLUSION

Up to date, over the counter sleep aiding agents have been introduced. St. John's wort (*Pipericum perforatum*), Kava kava (*Piper methysticum*), Valerian (*Valerinana officinalis*) and Passion flower (*Passiflora incameta*) and other herbs, which have been used for the treatment of insomnia become popular as an alternative medicine. Natural products which have been proven to be effective for sleeping from our laboratory are reviewed. However, preclinical and clinical studies are needed to evaluate the precise effects of natural products for the treatment of insomnia.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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