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Sleep promoting potential of low dose α-Asarone in rat model

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α-Asarone (PubChem CID: 636822)
Tween80 (PubChem CID: 5281955)
Midazolam 2 mg/kg (PubChem CID: 4192)

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ABSTRACT

Commonly used hypnotics have undesirable side-effects, especially during continuous usage. On the other hand, some herbal products, which are used for prolonged periods, are suggested to have a sleep inducing property, though the claims have not been validated scientifically. The hypnotic potential of α-Asarone, an active principle of *Acorus* species, was tested in the present study by first identifying the optimal dose of α-Asarone for improving sleep, followed by studies that evaluated the effect of repeated administration of this optimal dose for five days on sleep deprived rats. Of all the doses tested (2, 10, 40, 80 and 120 mg/kg), 10 mg/kg α-Asarone improved the quality of sleep, as indicated by an increased NREM bout duration, reduced arousal index, and decreased bout frequencies of NREM sleep and wakefulness. A marginal decrease in the hypothalamic and body temperatures was also observed. Higher doses, on the other hand, not only reduced the quantity and quality of sleep, but also produced hypothermia. In sleep deprived rats, administration of 10 mg/kg α-Asarone for five consecutive days improved the quality of sleep in contrast to the vehicle and a known hypnotic midazolam. Improvement in NREM sleep quality was observed when the difference between the hypothalamic and the body temperature was minimum. An enhanced association between NREM sleep bout duration and hypothalamic temperature was also observed after administration of 10 mg/kg α-Asarone. This comprehensive study is the first report on the hypnotic property of α-Asarone, which validates its potential to be considered for treatment of insomnia.

1. Introduction

Insomnia is one of the major health issues of the current era which is characterized by 'a persistent difficulty with sleep initiation, duration, consolidation, or quality that occurs despite adequate opportunity and circumstances for sleep, and results in some form of daytime impairment' (American Academy of Sleep Medicine, 2014). It is said that about one-third of the general population may be classified as insomniacs solely on the basis of difficulty initiating or maintaining sleep (Ohayon, 2002). Drugs like benzodiazepine and non-benzodiazepine are commonly used for the management of insomnia (Chouinard, 2004). However, their prolonged usage leads to several residual side effects like drug dependence, tolerance, rebound insomnia, muscle relaxation, hallucinations, depression and amnesia (Ashton, 1994; Chouinard, 2004; Stone et al., 2008). Consequently, there is a persistent need to find a safer hypnotic for the treatment of insomnia. Traditional medicines offer a wide range of herbs and herbal products with sedative properties (Gulia et al., 2017; Kumar and Gulia, 2016; Panara et al., 2013), some of which are used for prolonged periods without ill-effects. However, due to lack of scientific validation, these herbs and herbal products have still not found a place in the mainstream clinical practice in sleep medicine (Gulia et al., 2017; Kumar and Gulia, 2016). As a first step in this direction, it is necessary to substantiate scientifically the hypnotic potential of the active principles of many of these herbs in animal models.

α-Asarone, an active principle of *Acorus* species, is one such potential compound deserving pre-clinical evaluation of its hypnotic property (Gulia et al., 2017; Kumar and Gulia, 2016). Ability of α-Asarone for the induction of hypothermia and sedation was shown in seizure models and when it was co-administered with

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Abbreviations

S-W	Sleep-wakefulness
T_{hy}	Hypothalamic temperature
T_{body}	Body temperature
SD	Sleep deprivation
NREM	Non-Rapid Eye Movement
REM	Rapid Eye Movement

pentobarbital, reserpine and chlorpromazine (Dandiya and Menon, 1963, 1964; Menon and Dandiya, 1967; Pages et al., 2010). However, polysomnographic evaluation of the sleep inducing property of α-Asarone, along with the monitoring of hypothalamic (T_{hy}) and core body temperature (T_{body}) in free moving animals, is essential for studying the hypnotic property of this compound, and for understanding the mechanism involved in the modulation of sleep.

It is emphasized that sleep and thermoregulation are closely interrelated processes and are regulated by a common area in the brain namely the preoptic-anterior hypothalamic region (Gilbert et al., 2004; Mallick and Kumar, 2012). Thermal signals to the preoptic area and the basal forebrain not only modulate sleep but also ensure sleep homeostasis (John and Kumar, 1998; Mallick and Kumar, 2012; McGinty and Szymusiak, 2001). Subtle changes in the brain temperature, especially the T_{hy}, occur during changes in vigilant state from wake to non-rapid eye movement (NREM) sleep or rapid eye movement (REM) sleep. T_{hy} is decreased during NREM sleep and is increased during REM sleep and wakefulness (Heller and Glotzbach, 1976; Krueger and Takahashi, 1997; Obal, 1984; Parmeggiani, 1977). Lack of sleep or insomnia produces an increase in the brain and the core body temperature along with alteration in thermoregulation in humans and rats (Bonnet and Arand, 2010; Everson et al., 1989; Franken et al., 1991, 1993; Lack et al., 2008; Monroe, 1967; Obermeyer et al., 1991; Rechtschaffen et al., 1989; Romeijn et al., 2012; Shaw et al., 1997; van den Heuvel et al., 2006). Therefore, this study was aimed at investigating the hypnotic property of α-Asarone, by measuring sleep-wakefulness (S-W) along with T_{hy} and T_{body} in rats.

The hypnotic property of α-Asarone was investigated firstly by identifying the optimal dose of α-Asarone for promoting sleep and secondly by evaluating the effect of repeated injection of this dose for five days on rats subjected to sleep deprivation (SD). Hypnotic effect of the optimal dose administered prior to SD was investigated to test the potency of the drug to counter the SD-induced alteration in S-W, T_{hy} and T_{body}. Sleep deprivation study was conducted on three groups of rats, where the hypnotic property of the optimal dose of α-Asarone, was compared with vehicle and a positive control midazolam. Midazolam is a benzodiazepine primarily used as a hypnotic-sedative and an anxiolytic drug, which enhances the effect of the neurotransmitter gamma-aminobutyric acid (GABA) at the $GABA_A$ receptor (Bokonjic and Rosic, 1992; Lancel et al., 1996).

2. Materials and methods

2.1. Animals

The study was conducted on 20 adult male Wistar rats (250–350 g) housed in polystyrene cages, kept in controlled temperature (26 ± 1 °C) and light-dark schedule of 12 h (lights on at 6:00 h), with food and water provided *ad libitum*. All the surgeries and procedures employed in this study were approved by the Institutional Animal Ethics Committee of the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum, Kerala (SCT/IAEC-019/June/2012/77).

2.2. Surgical procedures

All the animals were chronically implanted under anaesthesia (Ketamine 60 mg/kg and Xylazine 5 mg/kg body weight, i.m.) to record S-W, T_{hy} and T_{body}. To assess S-W, screw electrodes (1 mm diameter) were stereotaxically implanted bilaterally (using Kopf small animal stereotaxic instrument) on the parietal cortex (AP: −3 mm and ML: 2 mm, as per rat brain atlas of Paxinos and Watson, 1997) for recording electroencephalogram (EEG) and stainless steel loop electrodes were sutured on either side of the nuchal muscles for electromyogram (EMG). To measure T_{hy}, a pre-calibrated K-type thermocouple was stereotaxically implanted, 1 mm anterior to the hypothalamus (AP: −0.26 mm, ML: 3 mm, DV: 6 mm) at an angle of 25°. The electrodes and the thermocouple were connected to an integrated circuit socket and the whole assembly was fixed on the skull using dental cement. For the assessment of T_{body}, a radio-transmitter (TA10TA-F40, Data Sciences International, USA) was implanted in the peritoneum for continuous telemetric monitoring of T_{body} without handling the animals (Fig. 1). The animals were given antibiotic and analgesic for five days and recording was initiated after the post-operative recovery period of two weeks.

2.3. Drug

α-Asarone (*trans*-1,2,4-Trimethoxy-5-(1-propenyl)benzene) and Tween80 (Polyoxyethylene (20) sorbitan monooleate) were obtained from Sigma-Aldrich Co. LLC. α-Asarone was freshly prepared in the base containing 5% Tween80 and normal saline. The base containing 5% Tween80 was taken as the vehicle. Midazolam 2 mg/kg was procured from the Neon Laboratories Ltd.

2.4. Experimental design

After recovery from surgical trauma, the rats were connected with head cables and left for overnight habituation in the recording chamber. To assess the pre-drug condition, three control recordings of S-W were taken simultaneously with both T_{hy} and T_{body} for 8 h (9:00 to 17:00 h).

2.4.1. Dose response study

After overnight habituation in the recording chamber with head cables connected, a baseline recording of all parameters was taken for 1 h (9:00 to 10:00 h). The rats (N = 5) were then given intraperitoneal injection of vehicle and various doses of α-Asarone (2, 10, 40, 80 and 120 mg/kg) at 10:00 h. S-W, T_{hy} and T_{body} were then recorded simultaneously for 7 h (10:00 to 17:00 h). Five doses of α-Asarone were tried on the same animal on different days using a counterbalanced repeated measure design. An interval of five days was given between each dose in order to prevent the effect of repeated drug administration. The behavior of the animals was also monitored for 7 h after drug administration.

2.4.2. Effect of repeated drug administration on SD rats

This study was conducted on 15 rats randomly distributed into three groups of 5 rats each. After giving an overnight habituation to the animals with the head cables connected, S-W, T_{hy} and T_{body} were simultaneously and continuously recorded in these rats for 9 h (8:00 to 17:00 h). After baseline recording of all parameters for 1 h (8:00 to 9:00 h), the rats received intra-peritoneal injection of vehicle or drug at 9:00 h followed by SD for 5 h (9:00 to 14:00 h) for five consecutive days by gentle handling method (Gulia et al., 2015; Radhakrishnan et al., 2015). The gentle handling technique based