Hypnotic Effect of *Salvia reuterana* Boiss for Treatment of Insomnia

Vaseghi G (Ph.D.)[1], Andalib S (Ph.D.)[2], Rabbani M (Ph.D.)[3]*, Sajjadi SE (Ph.D.)[4], Jafarian A (Ph.D.)[3]

1- Physiology Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
2- Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
3- Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
4- Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
* Corresponding author: Department of Pharmacology, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran
Tel.: +98-311-7922646, Fax: +98-311 - 6680011
Email: rabanim@yahoo.com

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Abstract

Background: Insomnia, which is difficulty in initiating and maintaining sleep, is a very common experience for many people. Considering the increasing interest in medicinal plants in the past decade, many plants such as *Coriandrum sativum, Salvia leriifolia, Salvia reuterana* and *Stachys lavanduli folia* have been used in Iranian traditional medicine to abate insomnia.

Objective: The present study was designed to investigate hypnotic effect of *Salvia reuterana* on male mice.

Methods: Ethanolic extract of *S. reuterana* was prepared. Five groups of 6 animals each were pretreated with vehicle, *Salvia* extract (50, 100 and 250 mg/kg; i.p.) or diazepam (0.5mg/kg; i.p.) 30 minutes before ketamine injection (100 mg/kg, i.p.).

Results: The latency and total sleeping times were recorded to determine the hypnotic effect of the extract. The results indicated that ethanolic extract of *S. reuterana*, reduced the latency time and induced the total sleeping time in a dose dependent manner, compared to saline group.

Conclusion: The present study suggests that *S. reuterana* produces hypnotic effect which can be evaluated clinically.

Keywords: Hypnotic effect, Insomnia, Ketamin-induced sleeping time, *Salvia reuterana*

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Introduction

Sleep has been one of the most deeply healing and revitalizing experiences known. Insomnia is defined as short and poor quality sleep that influences optimal human daytime functioning [1]. This lack of healthful and restful sleep is a common problem experienced by a host of people throughout the world. It has been reported that 10 to 20 percent of adults across cultures suffer from chronic insomnia [2]. Insomnia is a problem which leads the way to several nervous disorders, and its causes are many and varied. In fact, environmental factors [3], alcohol [4], caffeine [5], respiratory problems [6], renal failure [7], stress [8] and other psychological conditions [9] may give rise to insomnia. Sleep treatments vary from administration of artificial medicines to medicinal herbs. Synthetic hypnotics include a wide range of products such as benzodiazepines and non-benzodiazepines [10-12]. Synthetic sleeping medicines are successful in precluding insomnia. Serious drawbacks to these medicines, however, are day time fatigue [13], cognitive impairment [14] and physical dependence [15]. On the other hand, herbal hypnotics have been traditionally used to treat insomnia. Furthermore, side effects of synthetic sleep medicines have recently revived people's interest in utilizing medicinal herbs against insomnia. To date, many medicinal plants (e.g., Lavender [16], Passion flower [17], *California poppy* [18], *Valerian* [19], *Echium amoenum* [20], *Rosa damascene* [21], *Coriandrum sativum* [22], *Saffron stigma* [23], *Salvia reuterana* [24], *Salvia leriifolia* [25], *Stachys lavanduli folia* [26]) have been used for their hypnotic and sedative effects throughout the world.

Salvia genus has been administered for different medicinal purposes. It has been found all over the world, especially in tropical and temperate regions [27]. Genus *Salvia* is distributed in Iran by 58 species, of which 17 are endemic [28]. It has been reported that aerial parts of *Salvia officinalis* areused as hypoglycemic, *Salvia macrosiphon* as antimicrobial, *Salvia aegyptica* as anti-inflammatory, and *Salvia sclare* as tonic [29]. Some *Salvia* species also exertantioxidative effects [30].

S. reuterana, which is popularly called as Maryam Goli Esfahani in Persian, is distributed in center of Iran. It has been utilized in Iranian traditional medicine for its anxiolytic, sedative and hypnotic effects. Zargari demonstrated that *S. reuterana* possessed anxiolytic effect [28]. In our former study, we found that *Salvia reuterana* exerted anxiolytic and sedative effects [30]. To date, studies have dealt with anxiolytic and sedative effects of *S. reuterana*, although no research has tended to focus on its hypnotic effect. Therefore, the present study set out to determine whether *S. reuterana* exerts hypnotic effect.

Material and Methods

Preparation of plant

*S. reuterana*was collected from south west of Iran. The plant was identified at the Botany Department, Faculty of Sciences of Isfahan University.

Plant powder and ethanol at a ratio of 1/3 was utilized for extraction. The extract was thereafter dried in order to remove ethanol. The procedure was followed by solving the extract with Tween 80 and normal saline at a ratio of 1\11. The control included saline normal and Tween 80 at a ratio of 1\11.

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Animals

Male Syrian mice (Pasture Institute, Iran) with a weight of 25 to 30 grams were employed. Mice were housed under controlled environmental conditions with ambient temperature of 19°C, relative humidity of 55±10% and 12-h light/dark cycle. Standard pelleted chow and water were provided ad libitum.

Ketamine-induced sleeping time

Five groups of 6 animals each were pretreated with Normal Saline, salvia extract (50, 100 and 250 mg/kg) and diazepam (0.5 mg/kg) i.p. 30 minutes before ketamine injection (100 mg/kg, i.p.). The time between the injection of ketamine and the loss of the righting reflex was recorded as initiation of sleep. The time between the loss and regaining of the righting reflex was also recorded as the duration of sleep.

Statistical analyses were performed using the SPSS statistical software package. More precisely, data were analyzed by one-way ANOVA and Duncan post hoc test and $p < 0.05$ was considered significant.

Results

Figure 1 illustrates the time interval between injection of ketamine and onset of sleep in different groups (latency times). It was shown that the use of 100 and 250 mg/kg of the extract decreased the latency time of sleep to76±9 (sec) and 50±10 (sec), respectively.The latency time in control group (118 ± 2 (sec)) was significantly longer than in the group treated with 100 mg/kg of the extract ($p<0.05$) and the group treated with 250 mg/kg of the extract ($p<0.005$). Furthermore, the latency time of sleep in extract-treated mice with the dose of 250 mg/kg was comparable to latency time in diazepam-treated mice (52 ± 7 (sec)).

As can be decidedly noted in Figure 2, sleep duration in mice receiving 100 and 250 mg/kg of the extract increased to 2725 ± 226 (sec) and 3300 ± 300 (sec), respectively. Sleep duration in control group (1618 ± 113 (sec)) was significantly shorter than in the group treated with 100 mg/kg of the extract ($p<0.05$) andthe group treated with 250 mg/kg of the extract ($p<0.005$). Moreover, sleep duration in extract-treated mice (250 mg/kg) was similar to sleep duration in diazepam-treated group (3490 ± 271 (sec)).

Table 1 provides a breakdown pertaining to the summary of latency and duration of sleep in various groups.

Discussion

Returning to the question posed at the beginning of this study, it is now possible to state that *S. reuterana* produces hypnotic effect and can be utilized for treatment of insomnia.

We previously reported the anxiolytic and sedative effects of extract of *S. reuterana* at a dose of 100 mg/kg in mice using spontaneous locomotor activity and elevated plus maze tests. It was observed that the total locomotor activity count, which was measured during 15 minutes of the test, significantly declined in rats undergoing diazepam pretreatment and *Salvia reuterana* hydroalcoholic extract treatment. There was also a decline in locomotor activity at 5 minutes. An increase in the dose of the plant extract produced a higher sedative effect. In addition, we observed that the extract increased the percentage of time-spent and the percentage of arm entries in the open arms of the elevated plus-maze [31].

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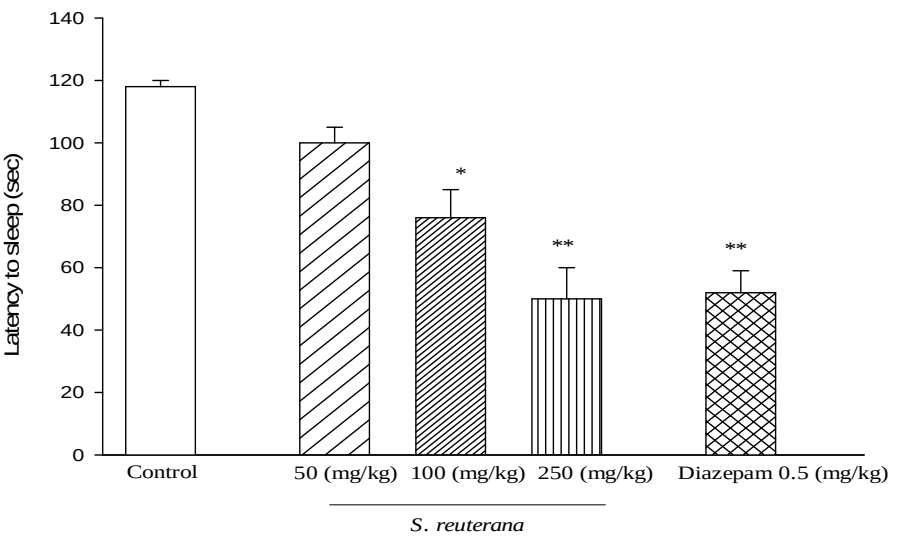

Figure 1- Effect of ethanolic extract of *S. reuterana* on latency to sleep induced by ketamine. There were six mice in each group and the results expressed as mean+ SEM.* p<0.05, ** p<0.005 compared with vehicle group

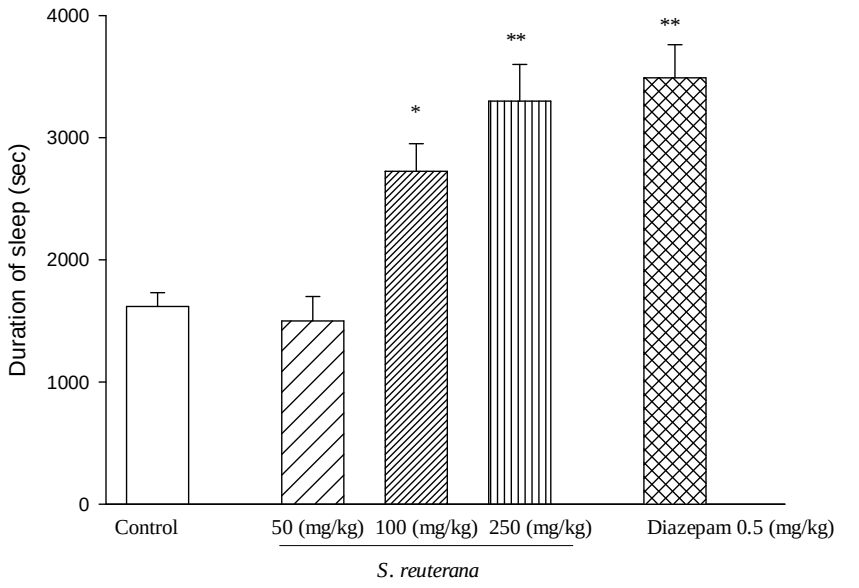

Figure 2- Effect of ethanolic extract of *S. reuterana* on duration of sleep induced by ketamine. There were six mice in each group and the results expressed as mean+ SEM. *p<0.05, **p<0.005 compared with vehicle group

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Table 1- Effect of extract of *S. reuterana* at 50, 100 mg/ kg doses on latency to sleep induced by Note. * p<0.05, ** p<0.005 compared with vehicle group

Groups	Dose	Latency to sleep (sec)	Duration of sleep (sec)
Control	-	118 ± 2	1618 ± 113
Diazepam	0.5 mg/kg	52 ± 7**	3490 ± 271**
S. reuterana	50 mg/kg	100 ± 5	1500 ± 200
	100 mg/kg	76 ± 9*	2725 ± 226*
	250 mg/kg	50 ± 10**	3300 ± 300**

Few studies have focused on constituents of *S. reuterana*. One study investigated the volatile constituents of *S. reuterana* and showed that there are 21 components in the oil of the plant; even so, the major constituents are (E)-β-Ocimene (32.3%), α-gurjunene (14.1%), germacrene-D (11.2%) and hexyl acetate (7.6%) [32]. Lee et al. demonstrated that *Salvia miltiorrhiza*, a Chinese herbal medicine, possessed Miltirone, as a major constituent exerting tranquilizing effect in mice [33]. They found that Miltirone inhibited the binding of [3H]flunitrazepam to central benzodiazepine receptor contributing to mechanism of action of GABA receptors (Lee 2003) [33]. In this regard, Perry et al. suggested another hypothesis that inhibition of acetylcholinesterase by chemical constituents of *S. lavandula efolia* brought about sedative and anxiolytic effects. These mechanisms can propose the hypnotic effect of plant [34]. Recent findings pertaining to hypnotic effects of medicinal herbs have led to a renewed interest in their utilization as alternatives for synthetic medicines. Many herbal hypnotics have been used in Iranian traditional medicine. Moreover, in our previous study, we observed that no hypnotic effect was produced by *Echium amoenum*, an herb that has been administered to abate insomniain Iran [20].

Althoughthe results of present research buttress the traditional idea about hypnotic effect of *S. reuterana*, a number of caveats need to be noted. Firstly, on the grounds that the constituents of this herb have not yet been completely worked out, it is recommended that further research be undertaken to determine its active constituents. Secondly, before commencement of clinical trials, further pharmacological and toxicological work needs to be done. Finally, considering the fact thatthis medicinal herb has not been approved in Iran, it is obliged togo through many approval processes paving the way from laboratory to market.

Conclusion

In conclusion, our data suggest that *S. reuterana* can decrease the onset of sleep and increase the duration of sleep.

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