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Original Article

Study of Sedative-Hypnotic Effects of *Aloe vera* L. Aqueous Extract through Behavioral Evaluations and EEG Recording in Rats

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Abstract

In this study, we investigated the sedative and hypnotic effects of the aqueous extract of Aloe vera on rats. In order to evaluate the overall hypnotic effects of the Aloe vera extract, open field and loss of righting reflex tests were primarily used. The sedative and hypnotic effects of the extract were then confirmed by detection of remarkable raise in the total sleeping time through analysis of electroencephalographic (EEG) recordings of animals. Analysis of the EEG recordings showed that there is concomitant change in Rapid Eye Movement (REM) and None Rapid Eye Movement (NREM) sleep in parallel with the prolonged total sleeping time. Results of the current research show that the extract has sedative-hypnotic effects on both functional and electrical activities of the brain.

Keywords: *Aloe vera*; Insomnia; Sedative-Hypnotic effects; Electroencephalography; Electromyography.

Introduction

Insomnia is a sleep disorder characterized by inability of efficiently falling into and staying asleep to restore normal states of energy and wakefulness (1, 2). Reportedly one-third of the general adult population (2) particularly females (3) experience insomnia at some point in their lives. Although the prevalence of insomnia estimates very largely based on the diagnostic methods (4), there is no doubt on the enormous economic impact of sleep disorders (5, 6). Insomnia may be associated with obesity (7), increased risk for metabolic syndrome (8), coronary artery disease (9, 10), depression (11), and anxiety (12, 13), as well as being a cause of concentration and memory problems (14, 15).

Given primary insomnia seems to be the most common diagnosis (2), intensive pharmacological treatment is inevitable in many patients. Although efficient therapeutics like benzodiazepines are available for insomnia, clinical applications are limited because of concerns about their potential abuse, dependence, and adverse effects (16). Behavioral therapies also have empirical evidence for relieving insomnia, however they have remained generally unemployed because of the time-intensive nature and need for expert trainees for effective application (17). Regarding the limitations and unfeasibility of existing therapies of insomnia, alternative and traditional

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medicine can be interesting as new treatment of insomnia.

Old materia medicas offer a variety of remedies for sleeping ailments, namely *Crocus sativus* (safron), *Egyptian lotus*, *Solanum nigrum* and *Aloe vera* (*A. vera*) (18). *Aloe vera* L. (*Aloe barbadensis* Miller) named as Sabr-e-zard (19), a succulent plant belonging to the Liliaceae family is amongst well known Iranian traditional medicine(20).

A. vera is one of the most widely used herbal medicines well known because of its local anti-inflammatory and healing properties. Besides being one of the most popular herbal medicines worldwide (21), it is also increasingly used in food industries (22). The clear gel isolated from the plant leaves, which has a variety of nutrients and bioactive molecules, is widely used in skin care, cosmetics and as "nutraceuticals" (23). Systemic consumption has also been empirically confirmed to improve a variety of health elements including immune system (24), angiogenesis (25), and gastrointestinal integrity (26).

A. vera therapeutic efficacies are relied on various bioactive compounds. Amylase and salicylates for instance render the extract as an anti-inflammatory and antibacterial agent (27). Sedative and hypnotic effects of *A. vera* have been reported in several Iranian and international old pharmacopoeias (18, 20 and 28). *A. vera* extract contains certain biochemical components such as flavonoids (29) and amino acids (30) which have been previously documented to affect sleep quality.

Regarding many surveys implying beneficial effects of *A. vera* in CNS diseases (31-33), the present work aims to find experimental support for the reported hypnotic effects of *A. vera* in traditional medicine. In order to do that, the effect of aqueous extract of *A. vera* leaves on locomotion and pentobarbital induced sleeping of rats was investigated. More details about influence of *A. vera* on the sleep architecture were obtained through investigation of Electroencephalogram (EEG) and Electromyogram (EMG) recordings of the animal.

Experimental

Plant material identification and extract preparation

A. vera leaves were collected in late summer from Qeshm Island in Persian Gulf which is located in south of Iran. The plant material were identified and authenticated by a botanical specialist at Herbarium Section of Department of Pharmacognosy, School of Pharmacy, Shahid Beheshti University of Medical Sciences (voucher number: 8105). *A. vera* extract was prepared according to the aqueous extraction method described in traditional herbal medicine (34). Accordingly, *A. vera* leaves were washed and grinded to small pieces, around 1cm in size. The grinded leaves (100g) were rinsed and mixed with water (1L) and were heated for an hour to obtain a viscose gel. The gel was cooled down in room temperature and filtered through a sieve with fine meshes. The extruded filtrate was then dried on a water bath yielding 6 g of dried extract.

Animals

Male Wistar rats weighting 250–300 g were housed in a temperature-controlled (22±2 °C) animal room on 12 h light/dark cycles 4-5 per cage with free access to food and water. Efforts were made to minimize the number of animals used and their suffering in according to the Ethical Committee for the use and care of laboratory animals of Shahid Beheshti University of Medical Sciences in compliance with the standards of the European Communities Council directive (86/609/EEC).

Loss of righting reflex

Animals assigned into pentobarbital-induced sleeping test received intraperitoneal (i.p.) injection of saline, diazepam (2mg/kg), or *A. vera* extract (50, 100, 200 mg/kg), 30 min before pentobarbital administration (40 mg/kg). The animals were then gently positioned on their back every 15 seconds and the onset of righting reflex loss was indicated while no righting movements in response to repeatedly positioning on their back were observed. Once the righting attempts were detected, the time period was recorded as duration of pentobarbital-induced sleep or loss of righting reflex.

Locomotion activity in rats

To examine sedative acute responses to *A. vera* extract, locomotion activity of the animals was evaluated using open field test.

Thirty minutes following i.p. injection of the extract (50, 100 and 200 mg/kg), animals were placed individually in the center of square arena of open field box (40×40×40 cm) and were allowed to explore freely the new environment. The locomotion activity of the animals were recorded and videotaped for 10 min using a digital camera installed on top of the arena. The distance travelled by each animal, named as total distance moved, was recorded as an indication of locomotion activity and was analyzed by Ethovision XT (Noldus, The Netherlands) software.

Surgical procedure

Chronically implanted electrodes were used to identify electrophysiological sleep-wake states of animals (35, 36). Following adequate anesthesia and analgesia achieved by ketamine (90 mg/kg) and xylazine (9 mg/ kg), four fine holes were drilled on different locations on the skull; two in lateral parietals (P1,2), one in lateral occipital (O) and the last one in frontal cortex (F). Stainless steel screws attached to insulated wire were implanted in the corresponding holes to serve as EEG reference electrode (F together with P1) or recording electrodes (O and P2). For EMG recordings, two wire electro-myographic electrodes were implanted in the neck musculature. All the EMG and EEG electrodes were connected to a miniature socket, which was cemented on skull with dental acrylic. After the surgical procedure, animals were allowed to recover for one week.

Sleep–wake state analysis of rats

Following implant recovery, the rats were prepared for electrophysiological recordings of EMG and EEG as described elsewhere (37). Briefly a shielded cable was plugged to the connector on the rat's head following diazepam (2 mg/kg) or *A. vera* extract (200 mg/kg) i.p. injection. The rats were then placed in the sleep-recording chamber which is an electrically shielded box designed for electrophysiological studies. EMG and EEG signals were recorded for four hours during the daytime (normally from 11:00 to 15:00) using Science Beam D3111 System of Biological Function. Recorded EEG (0-100 Hz) and EMG (20-2000 Hz) signals were amplified and analysed in 10s episodes using a custom-made program developed in MATLAB software (The Math Works Inc.) in conjunction with visual observation of the animal.

Each epoch was assigned to one of the following categories: wakefulness was scored based on the presence of fast low-voltage EEG concurrent with tonic high amplitude EMG. NREM was scored based on the presence of high-amplitude slow or spindle EEG activity in parallel with lower muscle tone and REM sleep was characterized by desynchronized EEG, absence of tonic EMG and occasional body twitches. The following variables were analysed as important sleep parameters: Total sleeping time (TST), REM and NREM sleep duration.

Statistical analysis

Locomotion activity, Sleep duration, and percent of REM and NREM sleep were analysed by one-way ANOVA. Data of each group were compared to a control group through Bonferroni›s multiple comparison tests and $p < 0.05$ was considered as significant difference.

Results and Discussion

Behavioral examination of sedative-hypnotic properties of A. vera aqueous extract

Investigation of pentobarbital-induced loss of righting reflex

As the present work essentially aims to investigate hypnosis in response to *A. vera* administration, changes in loss of righting reflex was considered to estimate hypnotic effects of the extract. Administration of the extract did not influence onset of pentobarbital induced sleeping (data are not shown here) but prolonged the representative loss of righting reflex as the main characteristic of hypnotic agents. As it is presented in Figure 1, administration of the extract (200 mg/kg) led to prolonged loss of righting reflex compared to the control group of animals. Prolongation of loss of righting reflex in the animals was statistically equal to that of the animals which had received diazepam (2 mg/kg) as the positive control.

Investigation of locomotion activity

While hypnotic properties of the extract were

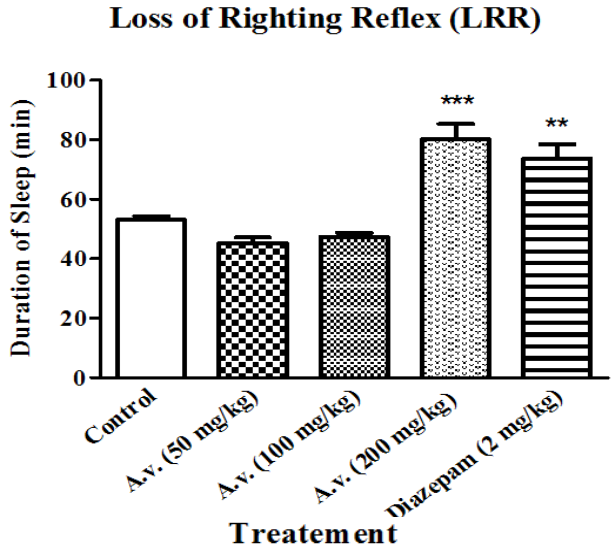

Figure 1. Aloe vera (*A. vera*) aqueous extract prolonged pentobarbital induced loss of righting reflex. Rats received pentobarbital (40 mg/kg, i.p.) 30 min following Aloe vera extract (50, 100, 200 mg/kg, i.p.) or diazepam (2 mg/kg, i.p.). *A. vera* hypnotic effects were evaluated based on increasing the sleeping time in test animals. Data are represented as mean ± SD (n=6). *** $p < 0.001$, ** $p < 0.01$ compared to control group.

primarily determined in loss of righting reflex examination, locomotion activity alteration was also considered as an index for the sedative effect. The open field results in conjunction with data obtained from loss of righting reflex test, confirmed sedative-hypnotic effects of the extract. Figure 2 shows significantly repressed locomotion activity of the animals after administration of the extract (100 and 200 mg/kg) probably as a result of its sedative effect. The results show that administration of the extract at dose of 200 mg/kg is as efficient as diazepam (2 mg/kg) in suppressing the locomotor activity of the animals.

Investigation of sleep parameters

In order to be able to define significant sedative-hypnotic effects for the extract, results of loss of righting reflex test, as a widely used screening test for hypnotic compounds, needs to be confirmed by complementary methods. Therefore EEG recording was performed to determine duration of awaking, NREM and REM sleep states. Figures 3 A, 3 B and 3 C represent changes in sleep pattern of the animals which received the *A. vera* extract (200 mg/kg) in four hours during daytime. Animals treated with *A. vera* had following sleep parameters: total sleep time [$F(2,15)=358$, $p < 0.05$], percent of REM sleep [$F(2,15)=15.5$, $p < 0.05$] and percent of NREM sleep [$F(2,15)=14$, $p < 0.05$]. Results of Bonferroni's post-test of EEG recording show that A.vera as well as diazepam increased the sleeping time ($p < 0.001$) and NREM sleep duration ($p < 0.001$) and decreased REM sleep ($p < 0.001$) compared to the control group.

Active pharmacological ingredients of *A. vera* , concentrated in the gel and rind of the plant leaves have been evidently shown to exert analgesic, anti-inflammatory, antioxidant and anticancer effects (38). Amongst identified therapeutic indications for *A. vera* in folk medicine, treatment of insomnia has not been addressed yet by any scientific experiment. The present work evaluates sedative and hypnotic effects of *A. vera* through performing open field as well as pentobarbital induced-sleeping prolongation, as screening tests, on rats. Whereas open field test is not specific for sedation–related behaviors, suppressed locomotion activity in conjunction with hypnosis determined by prolongation of loss of righting reflex in animals, may highlight sedative properties of the *A. vera* extract. Increase of total sleeping time observed

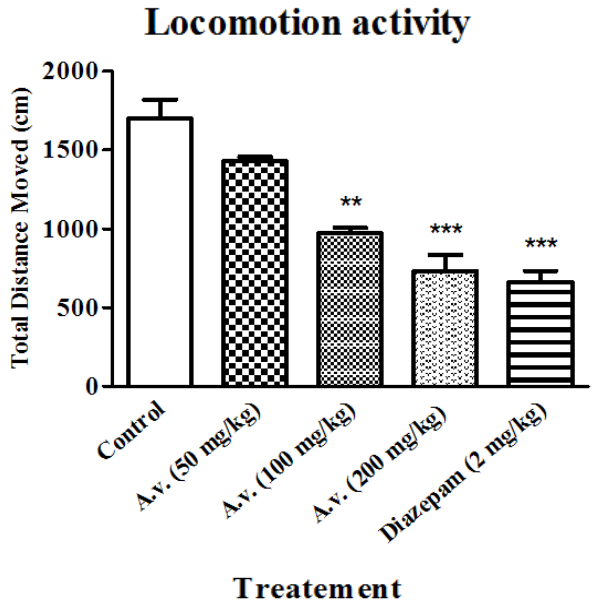

Figure 2. Aloe vera (*A. vera*) aqueous extract repressed animals' locomotor activity. 30 min after Aloe vera extract (50, 100, 200 mg/kg, i.p.) or diazepam (2 mg/kg, i.p.), animals were subjected to open field test and animals' total distance moved were compared as an indicative for locomotor activity. Data are represented as mean ± SD (n=6). *** $p < 0.001$, ** $p < 0.01$ compared to control group.

in investigations of EEG recordings of the same animals during the tests, could provide rational proof for the suppressed locomotion reported in our behavioral examinations.

To our knowledge, the only relevant survey by now has been a multicentre clinical open study on a topical moisturizer preparation containing *A. vera* extract for which 100% sleep improvement have been reported in subjects bearing atopic dermatitis (39). According to the study design however, this could be simply accounted for the relief from itching discomfort probably resulting from histamine release suppression by *A. vera* (26, 38) .

The essential characteristic of sleep is full reversal with efficient external stimuli. Therefore, virtual hypnotic impact of bioactive compounds could be accurately evaluated by EEG recording in normal, rather than drug induced sleeping animals. The EEG records of our day-time sleeping studies illustrate substantial electrophysiological effects of *A. vera* on sleep length that seemingly were efficient enough to produce the behavioral responses we observed in open field and loss of righting reflex test.

Besides prolongation of total sleeping time, *A. vera* administration led to a significant shift toward more NREM sleep. Such alterations in REM and NREM sleep parameters may provide useful data about outcomes of certain therapeutics. REM and NREM sleep are two distinct stages which are different not only in cerebral electrophysiological status but also in simultaneous resting muscular tonus. That is whilst thought-like mental activity takes place during NREM sleep. REM sleep is mostly associated with hallucinations concurrent with muscular paralysis. Several investigations on memory performance have also revealed that REM sleep contributes to consolidation of procedural memory (40) while NREM improves declarative memory (41).

The hypnotic activity of herbal medicines has been frequently attributed to different phytochemicals compounds such as flavonoids and saponines (29, 42). In the case of protein rich plants however, presence of certain amino acids may be of prominent importance (43, 44).

Versatile non-amino acid neurotransmitters such as acetylcholine and catecholamine are involved in governing normal sleep quality. That is centrally acting anticholinergic, dopaminergic, noradrenergic, and serotonergic agents cause a decrease in duration and density of REM sleep (45,

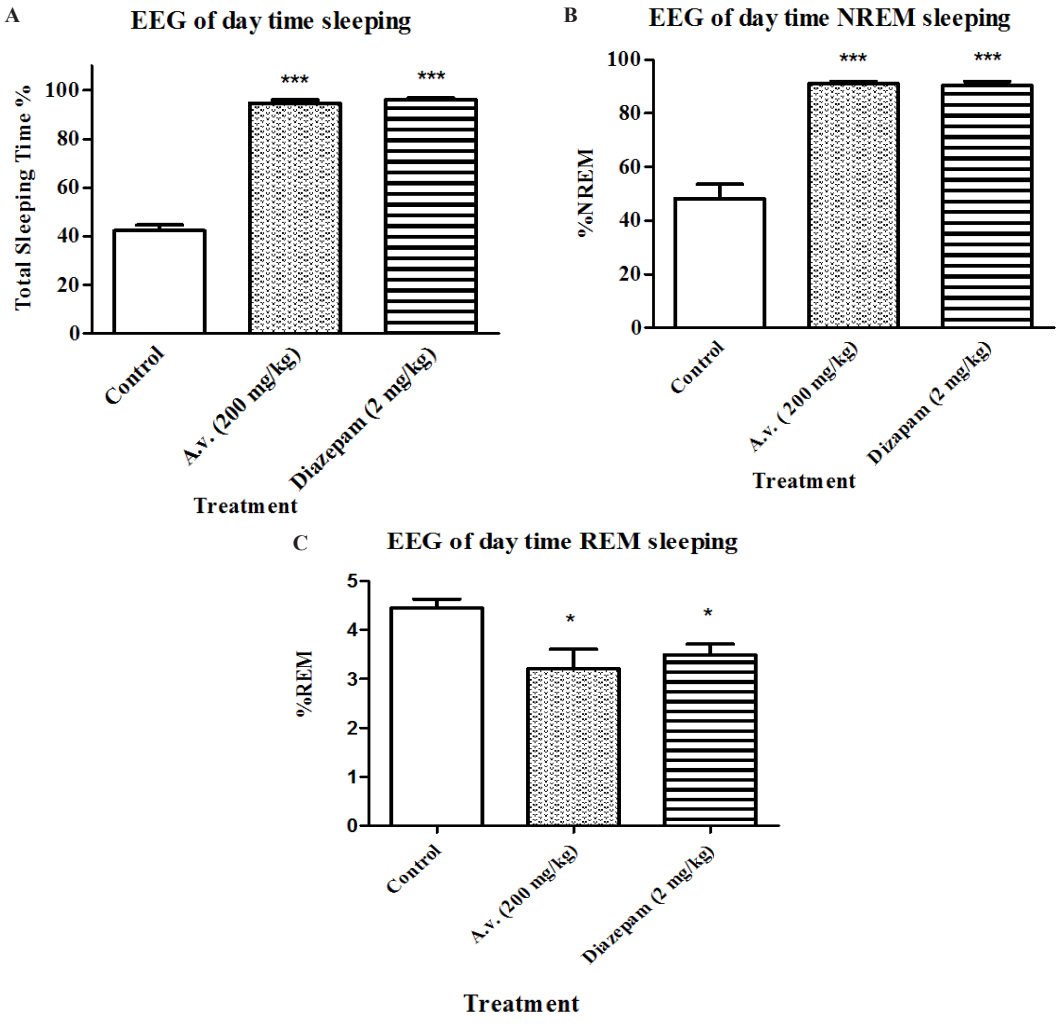

Figure 3. Impact of Aloe vera (*A. vera*) extract administration on electroencephalographic architecture of sleep. Day-time EEG and EMG recordings were conducted in freely moving rats following 30 min post Aloe vera or diazepam administration for 4 h. Accordingly, Aloe vera aqueous extract (200 mg/kg, i.p.) prolonged total sleeping time (A) as well as NREM sleep (B) while reduced sleeping time spent in REM (C). Data are represented as mean ± SD (n=6). * $p < 0.05$, ***$p < 0.001$ compared to control group.

46). Recent evidence has elucidated significant changes in cerebral neurotransmitters in mice treated with *A. vera* extract of which diminished levels of nore-epinephrine and serotonin are conspicuous (47). Regardless of probable impact on sleep parameters, we postulated such alterations might not apply to our set of experiments in which no long-term dosing protocols were included.

A. vera may be expected to elevate acetylcholine levels based on some reports implying its choline-esterase inhibition property (48). It has been shown that REM sleep duration is decreased by central cholinergic system augmentation and parasympathetic tone is dominant in NREM sleep (49, 50). The observed changes in REM and NREM sleep can be partly explained by presence of compounds with anti-choline-esterase activity in *A. vera*. The implication of any of the mentioned neurotransmitters however, needs further elucidation as our experiments did not include any contributing examination.

Conclusion

Several investigations have provided experimental evidences for CNS-ailments that have been traditionally claimed to be improved by *A. vera* administration of which convulsion

(51), cerebral ischemia (25) and multiple sclerosis (33) seemingly are the foremost ones. Present work provides positive evidences which support sedative and hypnotic effects of *A. vera* extract obtained by corresponding traditional method described in folk medicine. While investigating the corresponding properties of cold-dried extracts seem extremely intriguing for further works, our results also remained a question whether *A. vera* exerts ameliorating effects in the context of insomnia in human.

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