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Original Article

Evaluation of Pistachio Extract Standardized to 1% Melatonin on Pentobarbital Sodium-Induced Sleep in Swiss Albino Mice

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ABSTRACT

Background: Pistachios are rich in melatonin, vitamins and antioxidants, which are known to have potential sedative and hypnotic properties. Previous studies suggest that melatonin, a hormone involved in regulating the sleep-wake cycle, may enhance sleep quality. However, the sleep-inducing effects of pistachio extract combined with melatonin have not been extensively studied, especially in animal models. **Objectives:** The objective of this study was to evaluate the sleep-inducing effects of Pistachio extract containing 1% melatonin on pentobarbital-induced sleep-in mice. **Materials and Methods:** Pistachios were extracted using ethanol in a Soxhlet apparatus. The extract was then formulated into doses of 100 mg/kg, 200 mg/kg and 300 mg/kg body weight, each containing 1% melatonin. Sleep was induced in mice by administering a pentobarbital injection at 30 mg/kg body weight. The sleep onset time and total sleep duration were measured across different experimental groups. **Results:** The results demonstrated significant sleep-enhancing effects in the experimental groups. Group III (100 mg/kg pistachio extract with 1% melatonin) showed a reduced sleep onset time of 6.13±1.05 min and prolonged total sleep duration to 93.4±4.48 min. Group IV (200 mg/kg pistachio extract with 1% melatonin) exhibited a sleep onset time of 5.89±1.2 min and a sleep duration of 104.67±4.8min, while Group V (300 mg/kg pistachio extract with 1% melatonin) demonstrated a sleep onset time of 5.58±1.4 min and a sleep duration of 109.48±4.66 min. **Conclusion:** All three experimental groups demonstrated significant, dose-dependent sedative and hypnotic effects, significantly reducing sleep latency and increasing sleep duration. These findings suggest that pistachio extract, rich in melatonin, may effectively enhance sedative effects and improve sleep quality by reducing the time required to fall asleep and extending sleep duration.

Keywords: Melatonin, Pistachio, Sleep duration Sleep on set time, Sleep.

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INTRODUCTION

Sleep is a vital physiological process in shaping an individual's health and well-being.[1] However, inadequate sleep architecture results to sleep disorders like; obesity, heart diseases, obstructive sleep apnea, parasomnias, narcolepsy, insomnia and sleep behaviour disorder.[2] Physical, psychological, physiological and environmental factors affect sleep quantity and quality.[3] Roughly one-third of the population may be affected by a persistent sleep disturbance.[4] Subjective sleep disorder is the inability to start or sustain sleep, which is frequently non-refreshing or of poor quality and quantity.[5] Benzodiazepines and Benzodiazepines-like medications are commonly prescribed for these disorders despite the adverse reactions like drug dependence, increased tolerance, worsened insomnia upon discontinuation and amnesia.[6,7] Efforts are being made to explore treatments for insomnia from benzodiazepines to safer alternative treatments.[8] Development of an alternative medication requires consideration of numerous factors contributing to these disorders including diet, physical, medical, environmental, mental and genetic predispositions related to age.[9] Dietary practices are particularly important since they affect the sleep patterns. Specific diets and nutrients may affect sleep amount and quality.

Pistachios are rich in minerals like potassium, magnesium, calcium and phosphorus, along with vitamins A, E, C, B (excluding B12), K and folate. They also contain lutein, zeaxanthin and phenolic compounds such as anthocyanins and flavonoids, with notable antioxidant properties. Pistachios have the highest phytosterol content among nuts, including

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stigmasterol, campesterol and β-sitosterol.[10] Originating in Persia, they contain compounds like melatonin, magnesium and B vitamins, which best to promote sleep.[11,12] Melatonin regulates the sleep and wake-up cycles, while magnesium aids in relaxation and improves sleep. B vitamins act as cofactors in the production of neurotransmitters that control the sleep patterns.[13] Pistachios are abundant in polyphenols, including sphingolipids, which contribute to various health benefits such as antimicrobial, antiproliferative, antioxidant, anti-inflammatory and stress-relieving effects.[14] There is currently no pharmacological evidence supporting sedative-hypnotic properties of pistachio extract, the current study was conducted to investigate the effects of pistachio extract standardized with 1% melatonin on pentobarbitone sodium induced hypnosis in Swiss albino mice.

MATERIALS AND METHODS

Drugs and Chemicals

Carboxy Methyl Cellulose Sodium (CMC) 0.5%, normal saline, ethanol, picric acid, diazepam tablets and pentobarbital sodium injection (30 mg/kg) was sourced from the local vendor and diazepam 1 mg/mL (dissolved in normal saline).

Plant Collection and Extraction

Pistachio nuts were collected from local vendor in Bangalore, India. After being cleaned and shelled, the seed kernels were subjected for extraction. A total of 250 g of kernels were ground into a coarse powder and subjected to extraction with 1.5 L of 90% ethanol for 2 hr using Soxhlet apparatus. The extraction was carried out three additional times using same solvent mixture. The resulting crude extract was filtered and filtrate was distilled under reduced pressure on a water bath at 75±5°C to yield oleoresin extract. It was then mixed with ethanol and water, allowed to settle overnight at 10-15°C. Following filtration, the residue was dried under vacuum for 2-3 hr. This method effectively extracts melatonin and tannins, making it a valuable ingredient for addressing sleep related health disorders.[15]

Formulation

The formulations of Pistachio extract with 1% Melatonin were prepared based on required amount of Pistachio extract.[16] Initially, Pistachio extract with 1% Melatonin was triturated in a beaker, after which an appropriate amount of vehicle (0.5% CMC) was added. The mixture was thoroughly combined and transferred into a volumetric flask. Final volume was adjusted with vehicle. To ensure homogeneity during administration, the formulations of the Pistachio extract 1% Melatonin were continuous stirred using a magnetic stirrer.[17]

Experimental Animals

The present study was conducted on animals in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Male Swiss albino mice aged 8-10 weeks and weighing between 20-25 g were used in the experiment (Approval No.: RR/IAEC/131-2024).

Animals were housed in a standard polypropylene cage with a stainless-steel top grill and sterile bedding material and have provision for pelleted food and drinking water. Uncontaminated fresh water and Normal chow diet were provided to all the animals throughout the experiment. The mice were maintained under the controlled conditions of temperature at 22±3°C and relative humidity of 30-70% with a 12-hr light/dark cycle.[18] The animals were divided into five groups containing 8 animals in each group.

Grouping of Animals

Group I animals received saline (vehicle 0.5% CMC) as a negative control, serving as a control group. Group II was administered with Diazepam (1 mg/kg b.wt) was administered as a standard group acting as positive control. Groups III, IV and V receiving doses of Pistachio extract with 1% Melatonin at 100, 200 and 300 mg/kg body weight, respectively, serving as treatment group. To evaluate the sleep-inducing effects of the Pistachio extract 1% melatonin, all animals underwent sleep induction test, following treatment phase of experiment.[19]

Sleep Induction Test

The treatment groups (Group III-V) were treated with Pistachio extract 1% Melatonin, while standard group (Group II) was treated with Diazepam (1 mg/kg b.wt) and control group (Group I) received saline (0.5% CMC as vehicle). Half an hour afterwards, Pentobarbital sodium, 30 mg per kg of body weight was given via Intra-Peritoneal (*i.p.*) route to each mouse in all groups except Group II, to induce sleep. The onset time of sleep was recorded for all the animals. After induction of sleep, mice were placed in the inverted position and time taken for them to return to their usual body position was recorded. The duration in between administration of dose and induction of sleep was recorded as latency time.[19,20]

Statistical analysis

All the data were statistically analyzed using GraphPad Prism Software (version 5.01). The results were presented as Mean±SD. To assess the significant differences between the treatment and standard groups, One-Way ANOVA followed by Dunnett's test was applied.

RESULTS

The sedative properties of the Pistachio extract containing 1% Melatonin was evaluated by measuring the decrease in sleep latency time (in minutes) and prolongation of pentobarbital sodium-induced sleep duration (in minutes).

In the control group treated with saline onset time of sleep occurred at 7.18±1.03 min as shown in Table 1 and Figure 1 Diazepam as a reference drug, successfully reduced sleep onset time to 4.23±1.10 min which was highly significant with $p<0.001$. Similarly Pistachio extract with 1% Melatonin at doses of 100, 200 and 300 mg/kg body weight could significantly ($p<0.05$) reduce the onset of sleep time to 6.13±1.05 min, 5.89±1.20 min and 5.58±1.4 min respectively.

As illustrated in Figure 2, the control group receiving saline (vehicle 0. 5% CMC) prior to Pentobarbital injection had sleep duration of 22.90±1.89 min. As postulated, the standard drug diazepam significantly increased duration of sleep (147.09±4.17 min) ($p<0.001$ as compared to the control group). Pistachio extract with 1% melatonin at doses of 100, 200 and 300 mg/kg body weight also significantly increased sleep duration to 93.4±4.48 min, 104.67±4.8 min and 109.48±4.66 min, respectively ($p<0.001$).

The results showed that (G-III) significantly reduced the sleep onset time to 6.13±1.05 min and prolonged the total sleep duration to 93.4±4.48 min. Pistachio extract with 1% Melatonin at 200 mg/kg (G-IV) reduced sleep onset to 5.89±1.20 min and extended sleep duration to 104.67±4.80 min. Pistachio extract with 1% melatonin at 300 mg/kg (G-V) significantly reduced sleep onset to 5.58±1.51 min and prolonged sleep duration to 109.48±4.66 min ($p<0.001$). Additionally, it decreased locomotor activity, indicating a sedative effect. It is hypothesized that Pistachio extract with 1% Melatonin at this dose may act as a GABA agonist, directly interacting with GABA binding sites, enhancing the sedative effects by promoting GABAergic transmission.

DISCUSSION

The present study investigated the sedative effects of Pistachio extract containing 1% Melatonin and the results demonstrated a significant dose-dependent reduction in sleep onset time and prolongation of sleep duration across all experimental groups receiving doses of 100 mg, 200 mg and 300 mg. Numerous studies have explored the potential benefits of pistachio consumption on sleep quality. A randomized controlled trial by Canudas S *et al*[21] stated that subjects who consumed pistachios as a snack saw a dramatic increase in sleep quality. The melatonin content in pistachios may be key factor to alter sleep patterns.[22] Consuming pistachios at bed time led to significant increase in melatonin levels, suggesting pistachios might aid in body's natural ability to promote sleep. Moreover, pistachios are rich in magnesium, which is known to promote relaxation and better sleep.[22]

Sedative and hypnotic drugs on their part are licensed for use in the treatment of anxiety, calming and sleep induction as well as sleep maintenance.[23,24] The use of pistachios to address CNS diseases such as Alzheimer's disease and epilepsy is attributed to their strong anti-inflammatory characteristics, antioxidants, flavonoids, vitamins and minerals. Pistachio extract with 1% melatonin indicated the effective dose of 100, 200 and 300 mg/kg body weight showed dose- effectiveness to decrease the sleep onset and to increase the total sleeping time.

Pistachio extract with 1% melatonin may function as a GABA agonist by enhancing GABA receptor affinity and reducing muscle movement, which contributes to sleep induction.[25,26] GABA receptors play a crucial role in inhibitory neurotransmission in the CNS and can be modulated or blocked by various clinically significant drugs, including benzodiazepines.[27] The present study's data suggest that the interaction of Pistachio extract

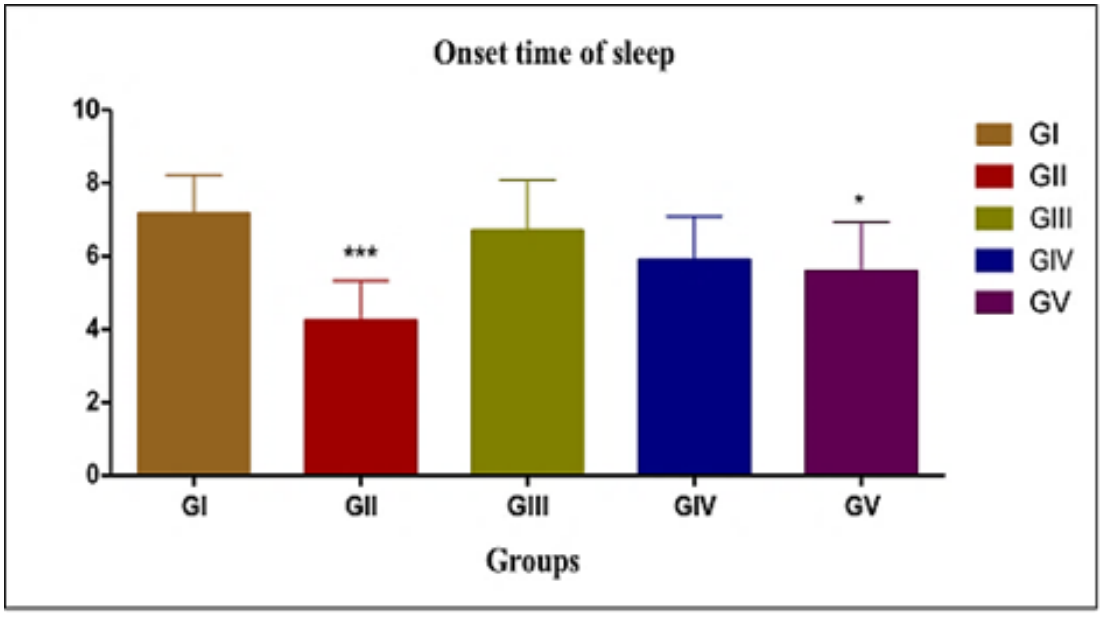

Figure 1: Effect of Pistachio extract 1% Melatonin on Onset time of Sleep (Minutes).

Table 1: Effect of Pistachio extract 1% Melatonin on Sleep induction test (Min).

Groups	Treatment	Mean Onset time of sleep (Min)	Mean Duration of sleep (Min)
Group-I	Control Saline	7.18±1.03	22.90±1.89
Group-II	Standard (Diazepam)	4.23±1.10***	147.09±4.17***
Group-III	Pistachio extract 1% Melatonin 100 mg	6.13±1.05	93.40±4.48***
Group-IV	Pistachio extract 1% Melatonin 200 mg	5.89±1.2	104.67±4.8***
Group-V	Pistachio extract 1% Melatonin 300 mg	5.58±1.4*	109.48±4.66***

Values were expressed as mean±SD (n=8), Statistical significance are compared between Control Saline (GI)versus other treatment groups (GII, GIII, GIV and GV) (* p Value < 0.05; ** p Value<0.001; *** p Value <0.0001).

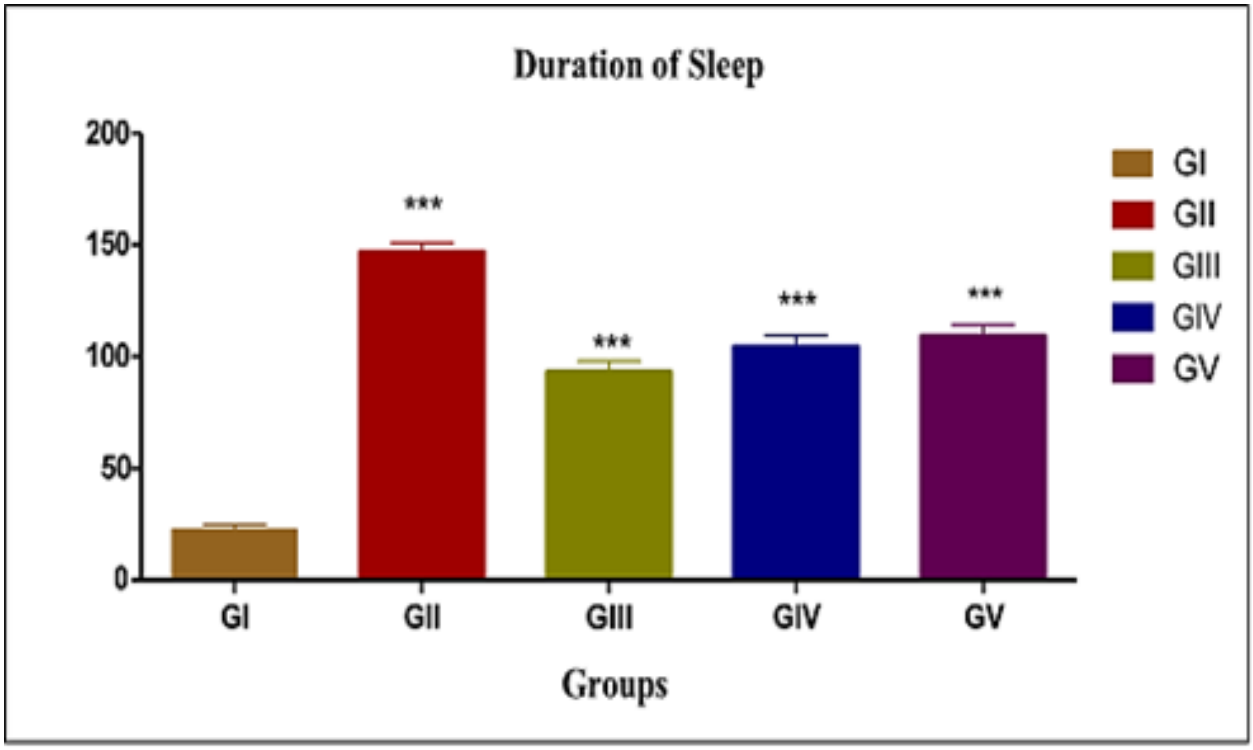

Figure 2: Effect of Pistachio extract 1% Melatonin on Duration of sleep (Min).

with 1% Melatonin enhances GABA receptor activity, indicating its effectiveness in GABAergic regulation during the observed sedative effects. Consequently, the sedative properties of this extract may stem from a GABAergic mechanism, similar to how Diazepam functions by increasing GABA receptor affinity.[27]

This study highlights the sedative effects of Pistachio extract with 1% Melatonin, characterizing it as a natural compound with GABAergic activity that reduces sleep onset time and increases total sleep duration. It may serve as a sedative and hypnotic agent by influencing GABA receptors. Additionally, the melatonin, magnesium and vitamin B content in pistachios[28,29] may help reduce stress and enhance sleep-wake cycles. However, further research is needed to clarify the mechanisms involved and determine the optimal dosage and timing for consumption.

CONCLUSION

Pistachios are a nutrient-abundant source of bioactive compounds that may promote better sleep. Including of pistachios in the diet could benefit to individuals experiencing poor sleep. All three test groups were found to have beneficial effects on sleep. Further clinical studies are necessary to validate these findings. Overall, pistachios can be considered as valuable addition to diet for enhancing sleep and overall health.

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CONFLICT OF INTEREST

The authors declare hat there is no conflict of interest.

FUNDING

No funding has been received for the current study.

ABBREVIATIONS

CMC: Carboxy Methyl Cellulose; **CPCSEA:** Committee for Purpose of Control and Supervision of Experiments on Animals; **CNS:** Central Nervous System; **GABA:** Gamma-Aminobutyric Acid.

AUTHORS CONTRIBUTION

The authors carried out entire research and involved in analysis of data from time to time.

ETHICAL APPROVAL

The experiments were conducted using male Swiss albino mice aged 8-10 weeks, with a body weight ranging from 20 to 25 g. The study protocol was approved by the Institutional Animal Ethical Committee of Radiant Research Services Pvt. Ltd., (Approval No.: RR/IAEC/131-2024).

CONSENT FOR PUBLICATION

This work is original and not published or under consideration in any other journal.

AVAILABILITY OF DATA AND MATERIAL

Will be made available on request.

SUMMARY

This study aimed to evaluate the sleep-inducing effects of Pistachio extract containing 1% melatonin in mice. Pistachio extract was prepared using ethanol and tested in three doses (100 mg/kg, 200 mg/kg and 300 mg/kg body weight). The effects were assessed by measuring sleep onset time and sleep duration following pentobarbital sodium-induced sleep. The results revealed that Pistachio extract significantly reduced sleep onset time and prolonged sleep duration in a dose-dependent manner. The group receiving the highest dose (300 mg/kg) showed a sleep onset time of 5.58±1.4 min and a sleep duration of 109.48±4.66 min, which were comparable to the effects of diazepam. Pistachios, rich in melatonin, magnesium and vitamins, appear to enhance sleep quality, potentially by promoting GABAergic activity, similar to the mechanism of action of benzodiazepines. These findings suggest that Pistachio extract with 1% melatonin may be a promising natural alternative for improving sleep and could be further explored for therapeutic use in sleep-related disorders.

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