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Sedative and hypnotic activity of the leaves of *Bijapura* (*Citrus medica* L.)

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Bijapura (*Citrus medica* L., family: Rutaceae) leaves powder when used along with honey is documented in ancient text to induce sleep and is useful in patients with insomnia. The purpose of the present research work was to investigate the sedative and hypnotic effects of *C. medica* leaves powder along with honey as *anupana* (adjuvant) in experimental animals. The effects of leaves powder on the locomotor activity of albino rats were evaluated using an open field test. The hypnotic effect was evaluated by potentiation of pentobarbital-induced sleep test and muscle relaxant activity by Rotarod test using swiss albino mice. Results were analyzed with one-way ANOVA and post-hoc Tukey's t-test with P <0.05 as significant. The leaves powder along with honey significantly (P <0.01 and P <0.05) reduced numbers of square crossed and locomotor activity in the Open field test when compared to control and vehicle control groups. It significantly (P <0.05) potentiated the pentobarbitone-induced sleep duration when compared to the control group. However, the leaves powder did not reduce or affect the latency of the fall-off time of mice in the Rotarod test. Vehicle as honey failed to produce significant effects when compared to the control group, whereas standard drugs as diazepam produced significant sedative, hypnotic, and muscle relaxant activity in albino mice. The result suggests that *C. medica* leaves powder has sedative and hypnotic activity without affecting the muscle tone/coordination in animals and thus, prove its traditional claim in insomnia.

Keywords: *Bijapura*, *Citrus medica*, Hypnotic activity, Insomnia, Muscle relaxant, Sedative activity.

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Introduction

Currently, sleep disorders have a relatively high prevalence and are a growing public health problem[1]. The most widely used medications for sleep disorders nowadays are benzodiazepines. However, the therapeutic uses of benzodiazepines are associated with untoward effects such as drug dependency, tolerance, rebound insomnia, amnesia, and psychomotor impairment[2]. Therefore, the exploration for new hypnotic and sedative agents with lesser side effects from traditional and complementary medicine is being carried out since the last decade. Medicinal plants have always been a good source to find safe remedies for human health problems. Lack of sufficient scientific data and a better understanding of the efficacy and safety of the herbs are major hurdles for its evidence-based medicine practice[3].

Ethnopharmacological approaches and traditional use of medicinal plants are significant contributions in reverse Pharmacology. Certain citrus species are used as sedatives to manage insomnia in traditional remedies[4] and several have been studied experimentally. Essential oil of *C. sinensis* exhibited CNS depression and sedative effects[5] and limonene enriched citrus essential oil has been reported to have anxiolytic effects in the light-dark test[6]. Essential oil of peel and hydroalcoholic extract of leaves from *Citrus aurantium* has been reported for its sedative activity in rodents[7] and *C. limon* exhibited anxiolytic and antidepressant effects in rats[8].

Citrus medica L. (Rutaceae), commonly known as citron (Sanskrit name: *Bijapura*), is used in Indian traditional medicine for many diseases. The main chemical compounds in leaf oil are citronellol, citronellal, limonene, citronellyl acetate, isopulegol, and linalool[9]. *C. medica* is well known for its reported activities such as antioxidant[10], antimicrobial[11], antihyperglycemic[12] and hypoglycemic[13] (insulin secretagogue bioactivity), anthelmintic[14], and estrogenic activity[15]. In Ayurvedic literature, leaves powder of *Bijapura* (*C. medica*) along with honey as *anupana* (adjuvant) is indicated to induce sleep in insomnia like disorders[16]. However, no pharmacological research exists to support the traditional usage of *C. medica* as a sleep aid. This prompted the author to evaluate sedative, hypnotic, and

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muscle relaxant activities of *C. medica* leaves powder in animal models.

Materials and Methods

Drugs and Chemicals

Leaves of *Bijapura* (*C. medica*), was collected from its natural habitat during May 2013, near Jamnagar (Latitude 22'28'59.37" N, Longitude 70'03'28.91" E), India and was authenticated by the taxonomist B. A. Jadeja, M.D. Science College, Saurashtra University, Porbandar, India. Leaves were washed, dried in shade, powdered, and stored in an airtight glass jar. The herbarium was prepared and deposited in the Pharmacognosy Laboratory of Institute with voucher specimen number Phm. 2013/14/ 6103. Honey was procured from Gujarat state forest development corporation Ltd. (Batch no. B-10 and Agmark replica no.WRR/H/01/12/c). It was multi floral in nature and was produced by dwarf honey bees found in natural forest. It was not crystallised, yellowish-brown in colour, sweet in flavour, and was stored at room temperature (25–30 °C) until used.

Animals

Charle's foster albino rats weighing between 200±20 g and Swiss albino mice of 30±5 g were used for the experiments. The animals were obtained from the Animal house attached to Institute. They were maintained under standard conditions of temperature (23±3 °C), relative humidity (50-60%) and 12 hours of light and dark cycles. The animals were acclimatized for one week before experiments with free access to standard laboratory feed and water *ad libitum*. Animals were fasted overnight prior to the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee (Ph.D./IAEC/13/2013/03) as per the guideline formulated by CPCSEA, India.

Dose

The human doses of *C. medica* leave and honey are considered as 3 and 5 g/day respectively. Considering the adult human dose, the dose for the rat and mice were calculated by extrapolating the human dose to animal dose based on the body surface area ratio[17].

Experimental protocols

Open field behaviour test

The Charles Foster albino rats of either sex were divided into four groups, each consisting of six rats per group. Group (I) kept as the control group, received distilled water (10 mL/kg, po) and Group (II) kept as the vehicle control group, received honey (450 mg/kg, po) in distilled water. Group (III) kept as the drug treated group, received leaves powder of *Bijapura* (*C. medica*) (270 mg/kg, po) with honey (450 mg/kg, po) in distilled water. Group (IV) kept as the standard drug-treated group, received Diazepam (4 mg/kg, po) in distilled water. The vehicle and drugs were administered once to albino rats of respective groups.

The study was conducted to evaluate the effect of the drug on spontaneous locomotor activity. One hour after drug administration, the animals were individually exposed to open field apparatus[18] (square box of 96×96 cm with a sidewall of 15 cm height having 36 squares). Each rat was placed in the arena for 10 minutes and activity was observed. The parameters recorded were the number of squares crossed (index of locomotor activity), number of rearing, freezing time (duration of immobility) and number of faecal pellets expelled.

Pentobarbital induced sleep test

Swiss albino mice were used to evaluate the efficacy of the drug on prolongation of pentobarbitone-induced sleeping time[19]. The albino mice of either sex were divided into four groups, each consisting of six mice per group. Group (I) kept as the control group, received distilled water (10 mL/kg, po) and Group (II) kept as the vehicle control group, received honey (650 mg/kg, po) in distilled water. Group (III) kept as the drug-treated group, received leaves powder of *Bijapura* (*C. medica*) (390 mg/kg, po) with honey (650 mg/kg, po) in distilled water. Group (IV) kept as the standard drug-treated group, received Diazepam (3 mg/kg, po) in distilled water. The vehicle and drugs were administered once to mice of respective groups. One hour after administration of test drugs, pentobarbitone sodium (45 mg/kg) was injected intraperitoneally. The mice were observed for the latency of onset of sleep and total duration of sleep[20].

Rota rod performance test

Evaluation of centrally acting skeletal muscle relaxant activity was evaluated using Rota rod performance in swiss albino mice. The selected albino mice were placed on a horizontal rotating metal rod having a diameter of 32 mm[21] and rotating at the rate of 20-22 RPM (round per minute). The animals that remained on Rotarod for two minutes or more after successive trials were included in the study. The mice were divided into four groups, each consisting of six mice as mentioned in the above protocol. The vehicle and drugs were administered once to mice of respective groups. One and three hours after

administration of test drugs, mice were again placed on the rotating rod in rotarod. The latency of fall off time from the rotating rod was noted at initial, after one and three hours of drug administration for each animal.

Statistical analysis

The data were analyzed using GraphPad PRISM 8.4.1 statistical software. Data were expressed as the Mean±SEM. The data generated during the study were analyzed with one-way ANOVA followed by Tukey's Post Hoc test was performed for inter-group comparisons for significant differences at P <0.05.

Results and Discussion

Literature of Ayurveda claims that the leaves powder of *C. medica* along with honey induces good sleep which suggests sedative and calming action among other properties. New drug discovery is considerably benefited by ethnopharmacological methods and reverse pharmacology on their traditional use. The attempts to search for novel plant-derived pharmacotherapies for psychiatric illness are progressively increasing in the past decade[22]. The present study was mainly carried out to evaluate the sedative of the *C. medica* leaves powder to provide a scientific basis to its traditional claim.

The open field test is an experimental test used to assay general locomotor activity levels and willingness to explore in animals (usually rodents) in scientific research[23].

In the open field apparatus, *C. medica* leaves powder along with honey significantly decreased the number of squares crossed when compared to the control group (P <0.01) and vehicle control group (P <0.05). Further, the drug produced a non-significant decrease in the number of rearing (vertical activity) and an increase in total freezing time when compared to control and vehicle control. Standard drug Diazepam produced a significant decrease in spontaneous motor activity and rearing while increasing the freezing time when compared to control and vehicle control (Table 1). The decrease in spontaneous locomotor activity by test drugs may lead to calming and sedation as a result of reduced excitability of the CNS[24].

Pentobarbital reduces the locomotor activity and increase the hypnosis in mice, revealed by lost of their righting reflex when they were laid on their backs[25]. *C. medica* leaves along with honey exerted significant potentiation of pentobarbitone-induced hypnosis effect by increasing total duration of sleep (P <0.05) and a non-significant decrease in latency of onset of sleep when compared to the control group in swiss albino mice. Vehicle as honey did not produce any significant effect on both the parameters. The standard treated group showed a significant decrease in latency of onset of sleep and increased the total duration of sleep when compared to the control and vehicle control group (Table 2). An earlier report suggests that the increase and decrease of pentobarbitone-induced sleep time can be a useful tool for examining the stimulatory or inhibitory effects on CNS, especially for investigating influences on $GABA_A$-ergic systems in CNS[22].

In rotarod performance, the difference in the latency of fall off time from the rod between the control and treated groups was considered as an index of muscle relaxation. The skeletal muscle relaxation together with calming effect also reduces anxiety and tension[26]. Locomotor activity and muscle coordination are the indicators of alertness and muscle relaxation[27]. In the present study, *C. medica* leaves powder along with honey treated group and vehicle control group did not show a significant reduction in latency of fall off time of mice from rotating rod in rotarod apparatus after one hour and three hours when compared to the control group. Diazepam treated group showed a significant decrease in the fall of time of mice from rotating rod when compared to control and vehicle control groups (P <0.001) (Table 3). The result of the present study suggests that *C. medica* leaves powder along with honey do not possess

Table 1 — Effect of test drugs on albino rats in open field test

Groups	No. of square crossed	No. of Rearing	Total freezing time (sec)
Control	162.2±6.67	28.33±5.17	12.5±3.04
Vehicle control	143.8±16.93	22.5±1.78	20.67±8.51
C. medica	81.67±15.16*$^{\#}$	16.17±3.98	36±12.42
Standard drug	18.4±5.69**$^{\#\#\#}$	2.2±0.73**$^{\#\#}$	497.2±31.33**$^{\#\#\#}$

Data: Mean±SEM (n=6); *P <0.01, **P <0.001, when compared to control group; $^{\#}P$ <0.05, $^{\#\#}P$ <0.01, $^{\#\#\#}P$ <0.001, when compared to vehicle control group (One-way ANOVA followed by Tukey's Post Hoc test)

Table 2 — Effect of test drugs on potentiation of pentobarbital-induced sleep in albino mice

Groups	Latency of onset of sleep (sec.)	Total sleep duration (min.)
Control	210.8±15.84	35±3.575
Vehicle control	198.5±11.07	46.7±4.86
C. medica	161.5±12.54	56.65±4.34*
Standard drug	108.5±7.82**$^{\#}$	110.4±8.13**$^{\#\#}$

Data: Mean±SEM (n=6); *P <0.01, **P <0.001, when compared to control group; $^{\#}P$ <0.05, $^{\#\#}P$ <0.001, when compared to vehicle control group (One-way ANOVA followed by Tukey's Post Hoc test)

Table 3 — Effect of *C. medica* leaves on swiss albino mice in rotarod performance test

Groups	Fall off time after 1 h (sec.)	Fall off after 3 h (sec.)
Control	218.4±24.48	231.6±20.48
Vehicle control	212±27.28	196.8±29.1
C. medica	206.7±17.11	191.2±16.87
Standard drug	16±6.06**	118.8±19.26*

Data: Mean±SEM (n=6); *P <0.05, **P <0.001, when compared to control group; $^{\#}P$ <0.001, when compared to vehicle control group (One-way ANOVA followed by Tukey's Post Hoc test)

muscle relaxant activity and has no effects on muscle tone/coordination in albino mice. Diazepam significantly exerted skeletal muscle relaxant activity by reducing the fall off time of mice in rotarod indicating the validity of protocol[26,28].

Anupana (adjuvant) is a unique concept in Ayurveda. To obtain the proper effects of drugs, the use of appropriate *anupana* along with specific drug therapy is equally important. It is a factor that helps in absorption, assimilation as well as potentiates the efficacy of the drug[29]. In the present study, honey alone in the vehicle control group did not exert any sedative or hypnotic-like effects but along with drug it potentiate the effects of *C. medica* which may substantiate the concept of *Anupana* in Ayurveda.

As per chemotaxonomy, closely related plants species may have a similarity in their chemical compositions. Earlier, CNS activity has been studied on other species of *Citrus* genus viz. *C. aurantium*, *C. limon*, and *C. maxima*. It has been reported earlier that extract and essential oil from *C. aurantium* L. possess sedative and anxiolytic activities[30]. It is opined that the sedative, as well as the anxiolytic effects of essential oil from *C. limon* possibly involve the $GABA_A$ receptor complex[31]. Essential oils are reported to have agonistic/facilitatory activities at $GABA_A$ receptor complex[32]. Moreover, a phytochemical study of *C. medica* leaves revealed the presence of essential oils like citronellal, citronellol, limonene, citronellyl acetate, isopulegol, and linalool[33]. Most sedative-hypnotics used in the treatment of insomnia target the $GABA_A$ receptors. Thus, essential oils might be responsible for sedative and hypnotic activities of *C. medica* leaves by acting on the $GABA_A$ receptor complex.

Conclusion

From the present study, it is concluded that *C. medica* leaves powder mixed with honey to possess a sedative and hypnotic effect, which is consistent with its traditional use. Additionally, there was no impairment in motor coordination in mice which indicates that the *C. medica* leaves powder is devoid of motor deficit.

Conflict of interest

The authors declare that there is no conflict of interest.

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