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ORIGINAL ARTICLE

# Effect of UP165, a *Zea mays* Leaf Extract Standardized for 6-methoxybenzoxazolinone, as Sleep Adjunct

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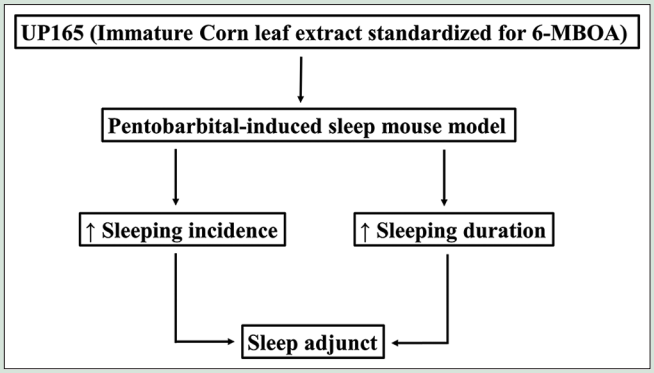
## ABSTRACT

**Background:** Sleep is a natural phenomenon essential for rejuvenating the body, promoting good health, upholding memory, performance, and maintaining overall health. Its deprivation is linked to the increased health risk that leads to poor quality of life and negative socioeconomic consequences. While behavioral techniques, such as improving sleep hygiene, are typically the first-line of intervention, pharmaceutical drugs are frequently used as adjuncts. However, for most, besides being too expensive, the long-term use of these drugs is marred by their severe adverse side effects and crippling dependency. As a result, significant numbers of patients are always in search for a safe and efficacious alternative from natural sources. **Materials and Methods:** We evaluated the effects of UP165, a *Zea mays* (commonly known as corn) leaf extract standardized for 6-methoxybenzoxazolinone content, on sleep latency and sleep time in pentobarbital-induced mouse sleep model. The extract was orally administered at 250 mg/kg (low dose), 500 mg/kg (mid dose), and 1000 mg/kg (high dose) daily for 32 days. The immediate impact of the extract on sleep was also assessed. **Results:** Increases of 11.6 ± 0.2 ($P$ = 0.008), 10.2 ± 2.4 ($P$ = 0.022), and 10.5 ± 0.9 ($P$ = 0.017) minutes in sleep time were observed for the 250, 500, and 1000 mg/kg UP165 treated mice compared to vehicle control, respectively. Up to 67% sleep latency incidence was observed for mice treated with UP165 compared to the 20% in the vehicle control group. UP165 showed no immediate drowsiness effect. No differences in baseline and end of study bodyweight were observed between groups. **Conclusion:** UP165 could be used as an adjunct for a sleep disorder.

**Key words:** 6-methoxybenzoxazolinone, anxiety, sleep disorder, stress, zea mays

## SUMMARY

- Sleep deprivation is linked to increased health risks that lead to poor quality of life and negative socioeconomic consequences
- The long-term uses of pharmaceutical sleep aid drugs are tarnished by their severe adverse side effects and crippling dependency
- UP165 is a *Zea mays* (commonly known as corn) leaf extract standardized for 6-methoxybenzoxazolinone content
- The effects of UP165 on sleep latency and sleep time were evaluated in pentobarbital-induced mouse sleep model administered orally at 250 mg/kg, 500 mg/kg, and 1000 mg/kg
- UP165 increased the sleep duration and incidence
- UP165 could be used as an adjunct for sleep disorder.




**Abbreviations used:** UP165-Maizinol, 6-MBOA-6-methoxybenzoxazolinone, SAMe-S-Adenosyl Methionine, GABA-gamma-aminobutyric acid, SK2 channel-Small conductance calcium-activated potassium channels.

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## INTRODUCTION

Sleep disturbance is one of the primary psychiatric disorders believed to be associated with stress, anxiety, and depression.[1] While its prevalence for adults with the chronic insomnia is approximately 10%–15%, other 30%–35% experience transient or infrequent insomnia.[2,3] Sleep deprivation is associated with cognitive impairment,[4] daytime sleepiness,[5] occupational hazard, loss of productivity, and traffic accidents.[6] A night of deprived sleep can impair performance equal to blood alcohol content considered illegal to drive (i.e., 0.10%).[7] In most cases, sleep disorders are not secondary to depression; for most, they often precede the depressive episodes and persist postremission, suggesting the significance of sleep disorder management as a priority for a better and successful clinical outcome of depression and anxiety.

The leading cause of insomnia, such as depression and anxiety, are the most common burdensome psychiatric disorders worldwide, posing significant adverse effects on activities of daily living for a considerable duration.[8,9] In the United States, they are among the prominent cause of disabilities, with almost 90% of persons with severe depressive symptoms reported to have difficulty with work, home, or social activities related to their symptoms.[10] Data from epidemiologic studies have also shown that abnormal sleep patterns predict lower life expectancy and that people with depression and insomnia are more likely to develop emotional disorders, substance abuse, and other adverse health consequences.[3] In clinical settings, improving sleep disorder in depressed insomnia patients caused improved health-related quality of life and depression severity.[11,12] Hence, the therapeutic intervention of sleep disorder and depression



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can be considered as the first line of defense in the war against these debilitating conditions.

The conventional pharmaceutical drugs are considered the primary choices and generally effective to provide momentary relief for insomnia and depression. However, for most, besides being too expensive, the long-term use of these drugs is marred by their severe adverse side effects and crippling dependency. As a result, significant numbers of patients are always in search for a safe and efficacious alternative from natural sources. For the past few decades, dietary supplement use among adults in the United States has increased significantly. Currently, more than half the US population is believed to use dietary supplements to improve (45%) or maintain (33%) overall health.[13,14] Although, far less is known about the use of dietary supplements by people with psychological disorders, studies show that 15%–36% take herbal and/or dietary supplements.[15,16]

Melatonin, a natural hypnotic produced by the pineal gland, is highly harmonized with the habitual hours of sleep. The daily onset of melatonin secretion is well correlated with the start of nocturnal sleepiness. There are compeling evidences documented describing the importance of melatonin both for the initiation and for the maintenance of sleep.[17] For instance, injection of pharmacological amounts of melatonin in rats during daytime increased total sleep time in a dose-dependent manner by increasing nonrapid eye movement sleep.[18] Low level of endogenous melatonin production has also been correlated with sleep disturbance in the elderly where it is corrected (significantly improved total sleep time and quality) following exogenous melatonin replacement therapy.[19] Thus, in our study, we used melatonin administered at oral doses of 1 mg/kg as a positive control.

To date, there are a few natural products on the market with sufficient scientific data in support of their psychotropic usage for good sleep and balanced mood. For example, the mood-enhancing activity of UP165, a *Zea mays* (commonly known as corn) leaf extract standardized for 6-methoxybenzoxazolinone (6-MBOA) content has been reported recently with equivalent efficacy to S-adenosyl-methionine (SAM-e) in a human clinical trial.[20] SAMe is a naturally occurring compound in the body that has demonstrated promising efficacy in the treatment of depression and anxiety.[21] When UP165 250 mg once per day was orally administered to patients with mild depression and anxiety for 8 weeks, statistically significant improvements in mood states were observed by improved Beck's Depression inventory II, the Beck Anxiety Inventory, and the Schwartz outcome scale-10. These clinical effects of UP165 are believed to be associated with the active component 6-MBOA which has (a) structural similarity to melatonin,[22] (b) ability to stimulate melatonin biosynthesis,[23] (c) weak β-adrenergic agonist activity with affinity for melatonin receptors,[24] and (d) believed to affect the level of melatonin precursor, serotonin.[25] Hence, there are reasonable structural and biological cause and effect networks among 6-MBOA, melatonin, and serotonin suggesting the possible application of UP165 for mood enhancement and sleep improvement.

In the present study, we evaluated the effect of UP165 on sleep latency and duration using melatonin as a positive control in a pentobarbital-induced sleep mouse model.

## MATERIALS AND METHODS

### Material

UP165 (lot# FP111612-01), a brown and odorless powder, was extracted from immature corn leaf (*Zea mays*) with ethanol and standardized to contain at least 0.2% 6-MBOA. UP165 was dissolved with sterile water. Melatonin was purchased from Sigma-Aldrich (M5250, Lot# 011 m1321V).

### Animals

BALB/C mice used (male, 18–22 g) were purchased from Beijing Hua Fu Kang biological technologies Inc. All mice were kept under specific pathogen-free conditions at Testing Center for the Functions of Health Foods, College of Arts and Science of Beijing Union University. Mice were provided with food (supplied by Beijing Hua Fu Kang Biological Technologies Inc.) and water *ad libitum*. A total of 150 mice were used in two sets of experiments in this report. Mice ($n$ = 15) were randomly assigned into five groups in each experiment. Groups include G1 = Vehicle control (sterile water), G2 = Positive control (melatonin; 1 mg/kg), G3 = Maizinol low dose (250 mg/kg), G4 = Maizinol middle dose (500 mg/kg), and G5 = Maizinol high dose (1000 mg/kg). Mice were gavaged once per day with the test materials suspended in sterile water at 0.1 ml/kg for 32 days. On the day of testing, 15 min after the last dose, mice from each group were administered with pentobarbital (Lot#: WS20101129, Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) at 26 mg/kg and 36 mg/kg for sleep latency and sleep time study, respectively, through intraperitoneal injection. Animals were observed during the latent period, defined as the time between pentobarbital administration to loss of the righting reflex, and the duration of sleeping time, defined as the time between the loss and recovery of the righting reflex.[26] Mice were observed for 60 min at the start of each study to monitor the direct impact of test compounds on sleep following a single oral administration.

## RESULTS

### Effect of UP165 on body weight

UP165 was administered orally to the mice at dose ranges of 250 mg/kg –1000 mg/kg daily for 32 days. Impact of treatment on weight gain as a side effect was monitored for each mouse. The average baseline and test day body weight were 20.6 ± 1.3 and 23.7 ± 1.4, respectively [Table 1]. No significant difference in body weight were observed for each treatment group either on day 0 (baseline) or on day 32 (end of the study).

### Acute effect of compounds on sleep

When naive mice are placed into a supine position, they instantaneously turn to the upright position. However, mice under the hypnotic dose of pentobarbital remain on the supine position for measurable amount of time. Sleeping was indexed as the disappearance of reflex to turnover to the right side, and mouse was considered asleep when the time to turn over to the side was over 30 s after mouse was treated with test substance. In the current study, none of the treatment groups induce sleep following a single oral administration [Table 2].

### Effect of UP165 on sleep time

Mice were injected with pentobarbital 15 min after the last dose of treatment to examine the effect of UP165 on sleep time. As shown in Figure 1, mice treated with the high dose of UP165 showed 11.6 ± 0.2 min (30.6 ± 9.4 vs. 42.2 ± 9.2, $P$ = 0.008) increase in sleep time compared to the vehicle control treated animals. Similarly, 10.2 ± 2.4 ($P$ = 0.022 vs. vehicle control) and 10.5 ± 0.9 ($P$ = 0.017 vs.

**Table 1:** Effect of UP165 on body weight

| Groups | Dose (mg/kg) | *n* | Sleep time | | Sleep latency | |
|---|---|---|---|---|---|---|
| | | | Initial (g) | End (g) | Initial (g) | End (g) |
| Vehicle control | 0 | 15 | 20.6±1.4 | 24.3±1.7 | 20.8±1.2 | 23.5±1.1 |
| UP165 | 250 | 15 | 20.5±1.4 | 24.2±0.9 | 20.6±0.8 | 23.3±1.2 |
| UP165 | 500 | 15 | 20.6±1.5 | 23.2±1.8 | 20.5±0.9 | 23.6±1.5 |
| UP165 | 1000 | 15 | 20.6±1.0 | 23.8±1.0 | 20.5±0.9 | 23.9±1.8 |
| Melatonin | 1 | 15 | 20.6±1.2 | 23.2±1.5 | 20.3±1.1 | 23.4±1.1 |

vehicle control) minutes increases in sleep time were observed for the 250 mg/kg and 500 mg/kg UP165, respectively. As expected, the reference compound, melatonin, showed 12.0 ± 1.3 ($P$ = 0.003 vs. vehicle control) minutes increase of sleep time compared to the vehicle control treated mice. UP165 orally administered at a dose level as low as 250 mg/kg resulted in statistically significant prolongation of sleep time in pentobarbital-induced mouse sleep model.

## Effect of UP165 on sleep latency

Latency of sleep is the number of animals with loss of the righting reflex in duration of time elapsed after pentobarbital administrations. The hypnotic subthreshold of pentobarbital sodium was determined and found to be 26 mg/kg, where 80%–90% of mice failed to show the loss of reflex to turn over to the right side. As a result, the subthreshold dose of pentobarbital was given to each mouse 15 min after the last dose of test materials to assess the effect of materials on sleep latency. Thirty minutes postpentobarbital injection, the number of mice with no reflex to turn over to the right side over the duration of 1 min was recorded. Data were reported as incidence of sleeping. As depicted in Table 3, 10 out of 15 mice (67%) for the high dose of UP165 and 12 out of 15 mice (80%) for the melatonin group were found to have shortened latency. These incidences were statistically significant for both the high dose of UP165 and melatonin compared to the vehicle control animals. In comparison, the incidence in the vehicle-treated group was only 20%. Positive trends (60% of mice both in the 250 and 500 mg/kg group) were also observed for the low and mid doses of UP165.

# DISCUSSION

In the present study, the sleep aid effects of different doses of UP165 and melatonin were assessed in mice with or without pentobarbital treatment for sleep time and latency. Data depicted here demonstrated that UP165 potentiated pentobarbital-induced sleep behaviors in mice. The duration required to fall asleep was also reduced as a result of UP165 treatment in mice at subhypnotic state. UP165 potentiated pentobarbital-induced sleep at all the dosages tested (250–1000 mg/kg). However, a single oral administration of UP165 at a dose level as high as 1000 mg/kg did not cause drowsiness or induce an immediate sleep. This suggests that there are differences in mechanisms of actions between UP165 and pentobarbital in induction of sleep. Pentobarbital, a short-acting barbiturate, binds at a distinct binding site associated with a Cl-ionophore at gamma-aminobutyric acid type A ($GABA_{(A)}$) receptors to produce their pharmacological sedative and hypnotic effects. On the other hand, the possible mechanism of actions for UP165 is discussed below.

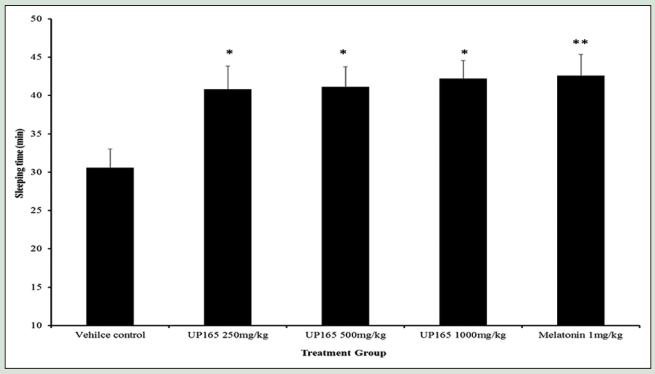


**Figure 1:** Effect of UP165 on sleep time: Mice were injected with pentobarbital 15 minutes after the last dose of treatment to examine the effect of UP165 on sleep time

Previously, Kalman *et al.* have shown the significant improvement of mood state of patients with mild anxiety and depression as a result of oral treatment of UP165 at 250 mg/day.[20] The authors made the plausible argument for the positive outcome, suggesting the structural similarity of melatonin and 6-MBOA and hence, the expected melatonin-like effect. The authors clearly laid out the fundamental correlation between melatonin and 6-MBOA and their impact on mood and depression. Melatonin level is known to impact mood states and sleep. There is enough evidence in the literature that corroborates the mood and mild anxiety and depression improvement activity of melatonin and its precursor serotonin in correlation with their levels in the body.[25,27-29] In the same study, the authors also described the possibility of 6-MBOA incurring its anti-anxiety and depression effect through induction of brain tryptophan hydroxylase (the rate-limiting enzyme in serotonin biosynthesis) and subsequent increase in serotonin levels.[30] Interestingly, besides regulating behavior and mood, serotonin also helps regulate sleep.[31] In addition, melatonin has been shown to play a key role in regulating sleep cycles and circadian rhythm.[32] Knowing the intertwined nature of anxiety/depression and sleep disturbance coupled with reported positive outcome one has over the other following treatment, there is a greater possibility for UP165 to produce a better sleep quality and therefore, a better mood state.[11,12,28,32] Substantiating our hypothesis, when moderately stressed patients were supplemented with monocot grass extracts for 4 weeks, 50% and 40% better sleep efficiency and quality were observed, respectively. In this randomized and double-blind placebo controlled study, the improved sleep quality and improved stress-related mood states were presumed likely due to 6-MBOA and its ability to influence serotonin levels.[33] In addition, using a mouse model of pentothal-induced sleep, the hypnotic activity of tryptophan (a precursor of serotonin and melatonin) was also evaluated and reported similar outcomes as what was observed in our preclinical sleep model study. Pentothal (Thiopental Sodium) thiobarbiturate is the sulfur analog of sodium pentobarbital with ultrashort-acting depressant activity to induce hypnosis. When tryptophan (8 mg/kg) was administered to mice in association with (GABA-a primary inhibitory neurotransmitter at 22 mg/kg), reduced sleep latency and prolonged sleep time were significantly in a mouse sleep model induced by Pentothal.[34] Collectively, these augmented evidence suggest the likely mechanism of action of UP165 in enhancing the state of mood, modulating the consequences of anxiety/depression and improving quality of sleep.

Moreover, it has also been well documented that compounds with anxiolytic activity to promote sleep induction due to their muscle relaxant

**Table 2:** Effect of UP165 on sleep following a single oral administration

| Groups | Dose (mg/kg) | *n* | Numbers of sleeping | Sleep time | *P* |
|---|---|---|---|---|---|
| Vehicle control | 0 | 15 | 0 | 0 | NA |
| UP165 | 250 | 15 | 0 | 0 | NA |
| UP165 | 500 | 15 | 0 | 0 | NA |
| UP165 | 1000 | 15 | 0 | 0 | NA |
| Melatonin | 1 | 15 | 0 | 0 | NA |

NA: Not available

**Table 3:** Effect of UP165 on incidence of sleep

| Groups | Dose (mg/kg) | *n* | Numbers of sleeping | Incidence (%) | *P* |
|---|---|---|---|---|---|
| Vehicle control | 0 | 15 | 3 | 20 | - |
| UP165 | 250 | 15 | 9 | 60 | 0.060 |
| UP165 | 500 | 15 | 9 | 60 | 0.060 |
| UP165 | 1000 | 15 | 10 | 67 | 0.025 |
| Melatonin | 1 | 15 | 12 | 80 | 0.003 |

properties. For example, 6-MBOA administered to rats at a dose level as low as 50 mg/kg produced decreased locomotor activities and hypothermia reflecting its muscle relaxant property.[35] These effects of 6-MBOA were the same in potency as chlorzoxazone (known centrally acting muscle relaxant drug) given at the same dose. However, 6-MBOA was nearly twice as potent as chlorzoxazone in potentiating thiopental-induced sleep. The mechanisms of action of these pharmacological activities of chlorzoxazone and structurally related compounds such as 6-MBOA were believed to be through activation of SK2 channels (small conductance calcium-activated potassium channels).[36,37] Although there was no direct objective measurement, the increased sleep latency and duration observed in the current study as a result of UP165 could partially be explained by the inherent ability of its active component to induce muscle relaxation and thus potentiate the effect of pentobarbital. This can be translated that, UP165 may provide a significant improvement in sleep quality and efficiency in humans. Due to its possible anxiolytic effect, there is a greater chance that UP165 may not induce acute sleep but rather prepares the body and mind to engage into sleep mode with a reasonable time frame leading to improved quality of sleep. In fact, in the current study, neither UP165 nor melatonin caused sleep induction following a single oral administration. Accordingly, unlike pharmaceutical sedatives that often lead to daytime drowsiness, the use of UP165 is not expected to induce sedative effects or daytime drowsiness.

While behavioral techniques, such as improving sleep hygiene are generally the first-line of intervention, numerous types of medications are frequently used as adjuncts. Prescribing sedating antidepressants such as the tricyclic antidepressants (e.g., amitriptyline and doxepin), the tetracyclic antidepressant (e.g., mirtazapine) and the serotonin antagonist and reuptake inhibitor (e.g., trazodone) are becoming the mainstream practice for patients with insomnia. Besides their daytime residual effect and long-term adverse consequences, the use of antidepressants in nondepressed patients raises ethical questions and remains controversial. As a result, natural sleep aids are widely used as alternatives to prescription drugs to improve the sleep quality and to avoid side-effects, including impaired cognitive function, tolerance, and dependence. However, there still are some side-effect concerns within the dietary supplement category. For example, while St. John's wort seems to be effective for the treatment of mild-to-moderate depression and SAMe for depression, both of these products have the potential to induce mania.[38] Similarly, anxiety for supplements containing serotonergic agents and stimulants,[39] and increased daytime sleepiness for melatonin have also been reported.[40] In this regard, with its historical safe usage track record supported by scientifically proven efficacy and safety data, UP165 could be a primary alternative for sleep aid and enhanced mood state without the associated side effects of melatonin.

## CONCLUSION

In summary, sleep disturbance is one of the most frequently observed symptoms associated with stress, anxiety, and depression. Improving sleep is associated with multiple favorable outcomes, including greater improvement in health-related quality of life and mood. Therefore, based on data depicted in this study and previously reported the effect of UP165 on depression, it is possible to infer that UP165 could potentially be used for better sleep and enhanced mood state. However, the suggested sleep aid segment of UP165 needs to be further validated in the human clinical trial.

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## Conflicts of interest

All authors are current Unigen employees; therefore, they have competing financial interests.

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