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CAENORHABDITIS ELEGANS IN SLEEP RESEARCH: COCOA AS A POTENTIAL MODULATOR IN KLEINE- LEVIN SYNDROME

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ABSTRACT

Kleine-Levin Syndrome (KLS) is a rare sleep disorder characterized by recurrent episodes of hypersomnia and behavioral disturbances. Its etiology remains unclear, and the lack of long- term effective treatments has driven the search for experimental models to elucidate its underlying pathophysiology and assess novel therapies. Among such models, *Caenorhabditis elegans* stands out due to its simple nervous system, short life cycle, and sleep-like behavioral states. In addition, cacao, rich in polyphenols and methylxanthines, has been shown to positively influence brain function and synaptic plasticity, suggesting a potential modulatory role in sleep disturbances. This review discusses the characteristics of KLS, the advantages of using *C. elegans* as a study model, and the possible role of cacao as a neurological modulator, proposing new therapeutic perspectives.

KEYWORDS: Kleine-Levin Syndrome, *Caenorhabditis elegans*, cacao, methylxanthines, polyphenols, sleep neurobiology.

1. INTRODUCTION

Kleine-Levin Syndrome (KLS) is a rare sleep disorder characterized by prolonged hypersomnia and behavioral changes.[1] Initially described in the 19th century and more clearly defined by Critchley and Hoffman in 1942[2], it predominantly affects adolescent males, with a prevalence of approximately 1 to 5 cases per million inhabitants.[3-6] In addition to hypersomnia, patients often experience hyperphagia, irritability, confusion, and other neuropsychiatric symptoms.[3,7]

The etiology of KLS remains unclear.[8] Various hypotheses suggest previous respiratory infections[9], genetic factors[10], and possible hypothalamic alterations, without consistent evidence of cerebral inflammation.[11] Relapses have also been reported following viral infections such as SARS-CoV-2, episodes of stress, alcohol and drug use, as well as sleep deprivation.[12-14]

In the absence of curative treatments, current therapeutic strategies focus on stimulants, antiepileptics, antidepressants, and other interventions, though results have been limited.[9,13] Recent trials, such as intranasal photobiomodulation and the use of methylene blue, show promising preliminary findings.[15]

This scenario underscores the need for robust experimental models to understand the biology of KLS and explore new therapeutic approaches. *Caenorhabditis elegans*, a nematode widely used in neurobiology, provides a valuable platform for studying sleep due to its simple nervous system, short life cycle, and well-characterized behaviors.[16-19] *C. elegans* can enter a Dauer state, a form of lethargy induced by adverse conditions[20-22], offering an opportunity to investigate sleep regulation and its disturbances.

Cacao, the main component of chocolate, contains polyphenols and methylxanthines with neuroactive properties capable of modulating brain function, memory, and synaptic plasticity.[23-25] These characteristics suggest its potential to improve sleep quality or alleviate symptoms of disorders such as KLS. This review analyzes the features of KLS, highlights the advantages of *C. elegans* as a study model, and examines the possible role of cacao.

2. Kleine-Levin Syndrome: Pathophysiology and Neurobiological Foundations

Kleine-Levin syndrome (KLS) is characterized by recurrent episodes of severe hypersomnia, accompanied by behavioral, cognitive, and eating disturbances.[8,26] During these episodes, which can last for days or weeks, patients may sleep up to 20 hours per day. They may also exhibit hyperphagia (with a preference for high-calorie foods), irritability, apathy, transient cognitive deficits, and spatiotemporal disorientation.[27-28] These episodes alternate with asymptomatic periods and typically resolve gradually in adulthood.[26]

The pathophysiology of KLS remains unknown. Studies suggest dysfunction in hypothalamic and thalamic circuits involved in sleep-wake regulation and cerebral metabolism.[28] Genetic, infectious, environmental, and possibly immunological factors may contribute to its manifestation.[26,27]

Although KLS is generally considered sporadic, emerging genetic evidence suggests a multifactorial, polygenic predisposition.[26-27] Variants in the TRANK1 gene, previously linked to neuropsychiatric disorders, may influence susceptibility.[29] However, large-scale genomic studies are necessary to confirm its role and elucidate the molecular pathways involved.

Current treatments — including stimulants, antiepileptics, antidepressants, lithium, and melatonin — tend to be palliative and offer limited short-term efficacy.[27-30] Non- pharmacological interventions, such as intranasal photobiomodulation, have shown promising results in isolated cases[31], but robust clinical trials are lacking. The absence of standardized, long-term therapies underscores the need for further research and a multidisciplinary approach.[26]

3. Animal Models in Sleep Research: The Role of *Caenorhabditis elegans*

Simple animal models have provided valuable insights into the regulation of sleep and its underlying mechanisms. Among these, *Caenorhabditis elegans* stands out due to its simplicity, reproducibility, and utility in the behavioral and neurobiological study of sleep.[32-35] Its nervous system, comprising only 302 neurons, combined with a simple anatomy, short lifespan, and transparent body, allows for the application of advanced genetic techniques, high-resolution microscopy, and detailed behavioral observations.[32,33] Under adverse environmental conditions, *C. elegans* larvae enter the Dauer state (L2d), characterized by reduced metabolism, arrested development, and extended longevity.[33] This adaptive and reversible diapause-like state has been considered a functional analogue of lethargic or sleep-related states, providing a useful framework for investigating the modulation of hypometabolic conditions.[33,36]

The ease of applying RNA interference, CRISPR/Cas9 gene editing, and fluorescent markers, along with the availability of mutant and transgenic strains, facilitates the identification of genes and pathways involved in sleep regulation.[37,34] These attributes make *C. elegans* a robust experimental platform for generating hypotheses about sleep physiology that can subsequently be validated in more complex models.

4. Cocoa as a Neurophysiological Modulator: Components and Mechanisms

Cocoa is rich in polyphenols (catechins, anthocyanidins, and proanthocyanidins) and methylxanthines (theobromine), compounds known for their neuroactive properties. These substances have been associated with improvements in cardiovascular health, metabolic function, mood, concentration, and cognitive performance.[40,42]

The Cocoa polyphenols present in cocoa possess antioxidant, anti-inflammatory, and vasodilatory properties, exerting positive effects on neuronal and synaptic function. Regular intake of these compounds may enhance attention, working memory, and processing speed.[40,43] Theobromine, the main methylxanthine in cocoa, exerts a moderate stimulant effect on the central nervous system, with a lower incidence of anxiety and insomnia compared to caffeine.[44,45] This profile suggests its potential usefulness in modulating alertness and sleep-wake cycles without significant adverse effects.

5. Therapeutic Perspectives: Cocoa in *C. elegans* as a Model for KLS

Employing *Caenorhabditis elegans* to evaluate the effects of cocoa on sleep-analogous states represents an innovative approach to exploring potential interventions for Kleine-Levin Syndrome (KLS). Although the nematode does not experience “sleep” in the strict sense, the Dauer stage exhibits functional similarities that facilitate the study of lethargy modulation, energy homeostasis, and functional recovery.[32,33]

Preliminary studies using cocoa extracts in post-Dauer larvae have shown improvements in mobility and growth, without significant changes in recovery time.[40] These findings suggest that cocoa may influence the organism’s overall physiological state, potentially through the modulation of pathways related to energy signaling, oxidative stress, and neuronal function.[38,40]

However, KLS is a complex and multifactorial disorder. Before proposing cocoa as a therapeutic intervention, it is necessary to:

- Characterize its bioactive components.
- Elucidate the molecular pathways involved.
- Conduct studies in more complex animal models and controlled clinical trials.[26-27]

Integrating simple models such as *C. elegans* with mammalian research and human clinical studies may guide the development of safe and effective therapies for KLS and other sleep disorders lacking long-term treatment options.

Conclusions and Future Perspectives

Despite its low prevalence, Kleine-Levin syndrome (KLS) poses significant challenges to sleep neuroscience, primarily due to its unclear etiology and the absence of curative treatments.[26,27] Its complexity — encompassing genetic, neurobiological, immunological, and environmental factors — further complicates the development of effective, long-lasting therapeutic strategies.[39]

The use of *Caenorhabditis elegans* as an experimental model provides a valuable platform for elucidating the underlying mechanisms of sleep and generating hypotheses about novel therapeutic pathways.[32,33] Cocoa, owing to its content of polyphenols and methylxanthines, shows potential for modulating brain function, although evidence supporting its direct role in KLS remains preliminary.[41]

Future research endeavors should focus on the detailed characterization of cocoa's active components, the elucidation of signaling pathways in more complex models, and the design of controlled clinical trials. By integrating genomic, neurobiological, nutritional, and neuroimaging approaches, the field may progress toward more effective, safer, and personalized interventions.[26,38]

In summary, a multidisciplinary and translational approach—combined with the validation of new therapeutic targets—will advance our understanding of KLS and enhance the clinical management of this and other sleep disorders.

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