Original Article

Electroencephalogram Correlates the Role of Ashwagandha in Sleep and Memory Promoting Effect

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ABSTRACT

Background: Ashwagandha (*Withania somnifera*), a known traditional medicine used in Ayurveda system of medicine, native to the India. Poor quality sleep always looked as serious complaint as it disrupts sleep. The natural phytoconstituents present in the ashwagandha believed to be adaptogen that helps the body to adapt stress by normalizing or correcting through balancing immune and neuroendocrine system. The mechanism that enhances sleep quality in the presence of ashwagandha is still unknown. Based on all available studies we investigated the effects of hydroalcoholic extract of ashwagandha effects on various bands of the electroencephalogram (EEG) to ascertain its role in behavior and sleep in rats. **Material and methods:** The present study explored in Wistar rats of either sex (n=12) used to record EEG in the presence of ashwagandha extracts (25mg/kg body weight). The previous studies revealed sleep promoting character of GABA very well documented in governing sleep and *Withania somnifera* known to posses mild to moderate hypnotic activity. We used conventional model for quantifying EEG in presence of ashwagandha extract and investigated its possible role in memory, behavior and sleep. *Withania somnifera* extract administered orally (25mg/kg) for electrophysiological recordings. Comparison made between post administration of extract with baseline recording employing vehicle and is considered as control. The changes in various bands of EEG noticed following administration of ashwagandha extract. **Results:** The *Withania somnifera* extract increased electrophysiological properties in delta ($p<0.001$) and gamma ($p<0.003$) bands and was found to be statistically significant. Statistically significant changes not observed in theta and alpha band power but beta band power was reduced in its influence ($p<0.013$). **Conclusion:** The present study revealed enhanced activity of the slow wave in presence of ashwagandha extract and divulged the details of its role in sleep and memory.

Key words: Ashwagandha, Data acquisition, Delta, Memory, Sleep, Frequency.

Submission Date: 10-08-2020;
Revision Date: 07-10-2020;
Accepted Date: 23-11-2020

DOI: 10.5530/ijper.55.1.20

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INTRODUCTION

Sleep documented as vital to the public health and shortage in quality and duration of sleep accountable for attentiveness, performance and health of the subject.[1] From an earlier evidence, the restorative component of the sleep aids brain and normal restoration of the body's metabolism.[2] It was scientifically proven that slow wave sleep has advantageous property on memory and learning.[3] Investigational studies have revealed inadequate amounts of slow wave sleep (SWS) caused weakened the alert system and lessening the cognitive performance. Enhanced SWS have many favorable effects on routine and daily activities including quality of the life along with physical and mental health.[4] All accessible sleeping medications haven't revealed to enhance the slow wave activity (SWA)[5] and despite increased sleep hours spent by a subject, observed the decreased SWA along with residual sleepiness in the daytime.[6] The goal of the present study is to explore the electroencephalographic analysis of the *Withania somnifera* Dunal (Ashwagandha, WS) based on the epoch analysis after administration of the extract of ashwagandha. Traditional system of medicine uses *Withania somnifera* Dunal and is comprehensively used in Ayurveda as a an constituent in many formulations for the treatment of various medical conditions and as a general tonic to improve health and helps in averting diseases.[7] For its adoptogenic properties it is also known as Indian ginseng and widely used in Ayurveda system of medicine in relieving stress.[8] The main active phytoconstituents, with anolides in *Withania somnifera* are responsible for the its adaptogenic and glucocorticoid-like effects.[9] In the face of modern medicines regardless of the advancement in treatment complementary and alternative medicine is found to be effective and safer for the treatment of various etiologies.[10] The modern life styles given rise to major threats in the form of central nervous system (CNS) disorders and are a major threat to the modern life and to the society. Efforts are being made to find a cure as well as to control different types of CNS disorders s millions of people are affected and medical sciences yet to find a cure for autism.[11] The complementary and alternative medical system at par to other already established therapies and demonstrated holistic approach in the treatment of the diseases with negligible side effects or lower than its synthetic counterpart.[12] Insomnia is a common complaints among middle aged populations along with other sleep disorder such as Restless Leg Syndrome (RLS) are common. Sleep impression commonly obtained using a combined impression of electrophysiological parameters.[13] The sleep pattern significantly disrupted in sleep deprived individuals.[14] Sleep related medical issue has already been documented the role of GABAergic system and withania root extract phytoconstituents known to posses mild to moderate hypnotic action[15] and drugs like benzodiazepines are reported exerts its effects by acting on this system.[16] The actions of the alkaloids (ashwagandholine, AG) extracted from the *Withania somnifera* roots studied for its actions on central nervous system,[17] in an another study involving the rat's brain investigated for its effects for brain cholinergic, glutamatergic and GABAergic receptors.[18] Slow Wave Sleep is part of deepest stage of non-rapid eye movement (NREM) sleep covers 10-30% of the total sleep time spent by an individual.[19] The SWS characterized by the high amplitude with low frequency wave and commonly known as delta waves. When suppression of the SWS is happened by the interruption either by arousal stimuli or due to sleep deprivation rebound phenomenon occurs during recovery of the sleep.[20,21] The medicines prescribed for sleep disorders have not shown to increase Slow Wave Activity (SWA).[22] In point of fact, the sleep medications lowers the Sleep Wave Activity and frequently associated with residual sleepiness in daytime, though sleep medicines enhances the sleep duration.[23] No report yet about *Withania* extract's electroencephalographic (EEG) analysis in normal rats, for that reason the effects of powder and extract of *Withania somnifera* investigated for possible changes observed in electroencephalogram.

MATERIALS AND METHODS

For the estimation of the electrical activity of the brain in Wistar rats of either sex employed for the conventional invasive method of EEG determination using extract of ashwagandha. Data acquisition system employed for the studies allows recording EEG signals generated in the brain of the rat comprised of connecting electrodes and chart recorders to record CNS changes. Data acquisition system uses graphical users interface and requires less time to set-up and include provision of change of channels and to plot the waveforms.

Electrode Implantation Surgery

Scalp sterilized with iodine-povidone solution after shaving rat's head, midline incision made with scalpel blade. Disinfected again with the hydrogen peroxide and to make cranial suture visible. Stereotaxic equipment used for electrode implantation at different points on the surface of the skull. Small electric drill machine used to lodge the screws in the skull, stainless steel screws used as electrodes measuring 2 mm diameter, 1 mm head size. For fixing the electrodes, parietal region at 2 mm fixed (AP – 2 mm; ML – 2 mm and 2 mm from bregma) on the skull bone to record the EEG from frontal and parietal cortices.

To fix the screw as a ground electrode, nasal bone selected. Three wires of 100mm thickness used to connect nine pin connector which was fixed on the rat head using dental acrylic cement. Care must be exercised that drilling should not damage the dura matter and after the drilling the holes, sterile saline is used to wash the drilled area. Screws are connected to the other end of the nine-pin connector using the soldering machine. Ketamine (72mg/kg, IP), xylazine (80mg / kg, IP) combination used as anesthesia agents, to prevent any respiratory distress atropine 8 mg/kg, IP administered. Iodine-povidone solution applied to control any post-operative infections and dexamethasone (1.5mg/kg) intraperitoneally administered for four days to reduce any inflammation. Post surgical procedure all the rats allowed to recover for 7-9 days before taking up for experimentation. Wooden EEG recording chamber

(12˝ × 12˝ × 11˝) is used to place the rats for EEG recording and the drug activity on various waves recorded in between 9 am to 2 pm through two channel MP45 data-acquisition unit and software AcqKnowledge 4.4, (Biopac System Inc, USA) by using high pass 5Hz and low pass 100Hz filters EEG signals for processing at 200/sec sampling rate. Recorded data of the EEG saved further for offline analysis. Four-second epoch considered for all five EEG bands and offline processing of the data using EEG spectral of 0 to 100Hz accessed by Fast Fourier Transforms (FFT). Electroencephalogram stated as percentage of the baseline recorded for any quantitative changes observed in EEG.

AcqKnowledge software (version 4.4, BIOPAC systems, Inc, the signals filtered 0.1 to 100 Hz with Power Spectrum Data (PSD) employed to analyze the various frequency bands of <4Hz (delta Band), 4-8Hz (theta band), 8-14 Hz (alpha Band), 14-30 Hz (beta band), > 30Hz (gamma band). Four seconds activity of the EEG recording averaged as spectra of power density and calculated to compare pharmacodynamic changes observed in all frequency bands. The in-built software algorithm used to calculate all five-power bands gamma, delta, theta, beta and alpha power, mean and standard deviation of absolute EEG power. For statistical analysis, the differences between each power bands considered. Ethanol extract of the ashwagandha (Gift sample from Sami Labs, Bengaluru) administered orally (25mg/kg) for electrophysiological recordings. With the permission of the Animal Ethics Committee (AEC), the experiments on the rats conducted at Jayamukhi College of Pharmacy, Narsampet. (JCP/IAEC/2014/01). Parietal region selected for the lodging disposable EEG electrodes as this region related to the brain activity.

Statistical Analysis

Paired sample *t*-test applied for before and after power bands of the EEG and used for calculation. EEG data analysis performed by using GraphPad Prism (Trial version) and applied paired *t*- test and the value $p<0.05$ considered as significant value.

RESULTS

The extract of ashwagandha had no effects on wakefulness of the rats, during entire amount of time rats did not sleep. Striking effects of ashwagandha extract noticed in delta band power, such noticeable effects observed in slow and fast bands. The recording of the control using vehicle was not produced any changes in delta band activity but extract effected in significant rise of delta band activity ($p<0.001$). In contrast, theta band slightly shortened in its power corresponding to baseline readings of the rats, but statistically insignificant. Favorable EEG recording acquired from freely moving rats within the enclosures. In all 12 rats, ashwagandha produced same type of variations in the EEG recordings with archetypal features, which distinguished the output of the EEG of the extract of the ashwagandha. The amplitude ranged from 0 to 0.25mV^2/Hz.

The various frequencies recorded for this experiment varied from 1Hz to 100Hz. The EEG epochs displayed as band frequency of gamma (30-100Hz), theta (4-8Hz), alpha (8-12 Hz) and beta (12-30Hz). Four seconds epochs are used to calculate Hertz at various places of the EEG recording, barring artifacts (Table 1). Paired '*t*' test applied for calculation by considering baseline data as control recording of 30 min. The maximum rise in frequency observed in delta band with 31.13% increase and found to be significant. The immediate next upsurge in band value observed in gamma with 3.89 % increase. No effect of extract exerted in theta band power of the electroencephalogram and found to be statistically insignificant, though there was slight decrease in the theta power (1.16 %). The steady changes in the delta band power with maximization of the power noticed in all 12 rats (Figure 1 and 2). The possible role of ashwagandha can be well explained by reduced beta band frequency to 9.58% to 9.55 %, this possibly explain drowsiness or its role as a remedial measure in sleeping disorder. The alpha band power value found to be statistically insignificant without much change in band power. This conventional method of EEG technique

Table 1: Effect of extract of ashwagandha on various frequency bands of the EEG in rats. *n*=12, Value represents Mean ± SD.

EEG band	Before Ashwagandha Extract Administration (Mean ± SD)	After Ashwagandha Extract Administration (Mean ± SD)	*P* value	Significance
Gamma	4.62 ±0.69	4.80±0.64	*P*<0.003	S (*P*<0.05)
Delta	3.95 ± 0.24	5.18 ±0.40	*P*< 0.001	S (*P*<0.05)
Theta	4.29 ±0.59	4.24±0.52	0.541	NS
Beta	4.59 ±0.69	4.15±0.32	*P*<0.013	S (*P*<0.05)
Alpha	4.11 ±0.25	4.09±0.15	0.706	NS

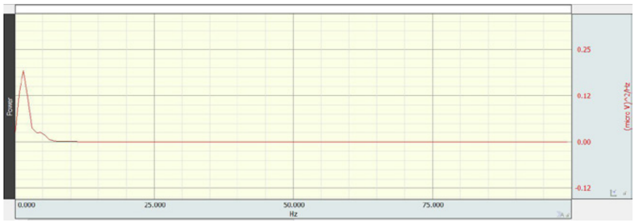

Figure 1: Changes in the frequency of delta band before administration of ashwagandha extract.

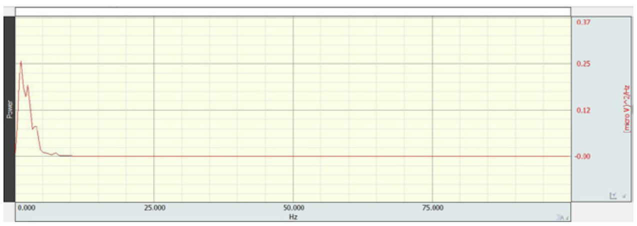

Figure 2: Changes in the frequency of delta band after administration of ashwagandha extract.

did not produce much change in theta band and extract of ashwagandha not exerted any action in alpha band power either.

DISCUSSION

The ashwagandha extract contain with anolides 7.3 % w/w and withaferine–A 1.1% w/w along with alkaloids 1.7% w/w. Revealed a prominent increment of delta and gamma band powers, this is a reflection of slow wave activity (SWA). This part of changes in increase within slow wave activity is an electrophysiological marker of sleep predisposition.[24] Sleep regulation is marked by the presence of slow waves of non rapid eye movement (NREM) sleep, this characteristic band noticed during the experiments. This is a prominent event occurs in EEG during sleep and happen and occur all through deepest stage of NREM sleep. These spontaneous oscillations consists of an upstate, with neurons firing erratically, which is equivalent to typical waking level followed by hyperpolarized state, wherein neurons fails to fire.[25] Many remedies potentiate slow wave activity, several efforts have been made and shown pivotal role of slow wave activity. This activity of slow wave acting on CNS mediated by facilitating GABAergic transmission. The isolation of ashwagandha, a bioactive glycowithanolides from roots of the plant in rats elicited pharmacological actions those like benzodiazepine, lorazepam for anxiety and imipramine for depression.[26] In a clinical study involving 30 subjects *Withania somnifera* enhanced sensorimotor function, auditory and mental ability, this study strengthens the role of ashwagandha in memory and concentration.[27] During the course of intensive learning, an increase in SWA observed in EEG power between 0.5 and 4 Hz during NREM sleep stage.[28,29] Brain slow waves are involved in helpful functions of sleep.[30] The role of slow waves in the formation of memory is extensively investigated[31] and declarative learning process shown that slow waves (<1Hz) are integrated.[32]

Effect on alpha band power in the presence of ashwagandha extract not observed. In a study conducted on patients with major depression, the selective serotonin reuptake inhibitors (SSRI) paroxetine elevated relative power of the slow wave delta and theta band power and reduced alpha band activity.[33] Similarly with *Withania somnifera* extract an increase in the delta power observed. This is also one of the reasons ashwagandha recommended as a mental tonic. In obsessive compulsive disorder patients shown a reduction in absolute power of delta and beta bands.[34] In a research study involving right lateralization for gamma band frequency indicated the role of gamma band in cognitive and behavior.[35] Following administration of extract of *Withania somnifera* a surge in gamma activity noticed, this confirms the role of ashwagandha in cognitive processing.

CONCLUSION

In conclusion, the present study revealed extract of ashwagandha having its effects on slow waves of the EEG observed in rats. Based on the current findings we distinguished potential role of ashwagandha in improving sleep quality in patients with insomnia and anxiety. The present study corroborates previous research findings that ashwagandha extract used as an effective sleep and anxiety aid and its cogent role in cognitive and memory functions.

ACKNOWLEDGEMENT

SVM is grateful to AICTE, New Delhi for receiving Grant under Research Promotion Scheme (RPS) (Ref. No. 8-125/RIFD/RPS/POLICY-3/2013-14) to carry out project work on ‘Development of Non-invasive EEG Technique in Animal Model’. SVM also wish to thank Dr. Sushil K. Jha, School of Life Sciences, Jawaharlal Nehru University, New Delhi and Shweta Tripathi, JNU for their timely help during the project.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

EEG: Electroencephalogram; **SWS:** Slow Wave Sleep; **SWA:** Slow Wave Activity; **CNS:** Central Nervous System; **RLS:** Restless Leg Syndrome; **NREM:** Non Rapid Eye Movement; **AP:** Anterior-Posterior; **ML:** Medial-Lateral; **FFT:** Fast Fourier Transforms; **PSD:** Power Spectrum Data; **AEC:** Animal Ethics Committee; **SSRI:** Selective Serotonin Reuptake Inhibitors.

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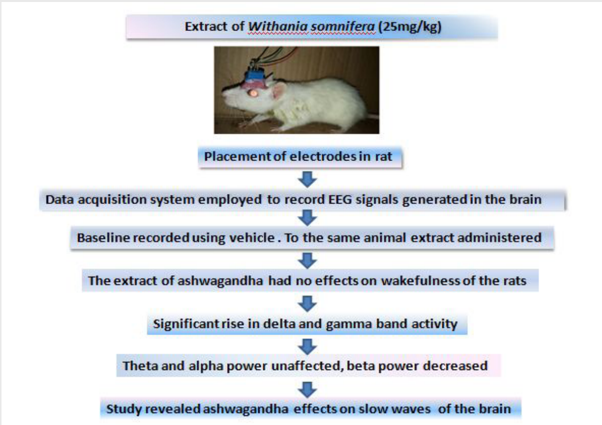
PICTORIAL ABSTRACT



SUMMARY

The research envisaged the possible role of ashwagandha, a known adaptogen in the management of poor quality sleep. The unknown mechanism that enhances the quality of sleep investigated using EEG to establish its role in sleep. The research ascertained the extract of ashwagandha increased electrophysiological properties of slow waves and revealed its role in sleep and memory.

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Cite this article: Basani M, Malothu R, Sinha RK, Murthy SV. Electroencephalogram Correlates the Role of Ashwagandha in Sleep and Memory Promoting Effect. Indian J of Pharmaceutical Education and Research. 2021;55(1):184-9.