Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.12.03.Art040

Hypolipidemic Effect of *Withania somnifera* in Sleep Deprivation Stress among Male *Wistar rats*

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Abstract

In view of growing evidence it seems that lack of sleep increases the risk for developing metabolic syndrome. IGF1 could mediate the atherosclerotic process by affecting the biomarkers of lipid metabolism. Withania somnifera (WS) also known as Ashwagandha is widely used for its medicinal properties. But no reports exist on its effect on lipid metabolism of sleep deprivation induced rats in relation to the tropical growth factor IGF-1. 24 male Wistar rats (120-150g) were divided into 4 groups with 6 animals in each (Group I - cage control, Group II - large platform control, Group III - sleep deprived & Group IV – WS treated sleep deprived (SD) rats. RT-PCR based mRNA expression analysis of Insulin like growth factor (IGF-1) in the cortex of the study groups was done. Concurrent protein expression analysis was carried out using western blot. Data was analysed by one-way ANOVA and Duncan's multiple range tests in SPSS software version 20. Our study demonstrated an inhibitory effect of sleep deprivation on IGF-I concomitant with increased serum TC, TG, LDLc & decreased HDLc levels and the changes on it correlated positively with withania somnifera treatment.

Keywords: *Insulin like growth factor –1 (IGF-1), Lipid metabolism, Sleep Deprivation (SD), Withania somnifera (WS).*

Introduction

In view of growing evidence, it seems that lack of sleep not only increases the risk for developing respiratory and cardiovascular disorders [1] but also claims metabolic syndrome [2] which is a cluster of conditions that increases the risk of stroke and diabetes in addition to heart disease. Dyslipidaemia, such as high levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and low levels of high-density lipoprotein cholesterol (HDL-C), increases the risk of cardiovascular disease [3]. Various epidemiological studies have suggested the complication of atherosclerosis and CVD in association with short sleep duration. Though the biomarkers of lipid

Received: 03.07.2024 ***Accepted: 20.08.2024*** ***Published on: 30.09.2024***
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metabolism (Total cholesterol, serum triglyceride, HDL-cholesterol & LDL-cholesterol) and sleep duration was investigated individually in those studies, the methods used to determine the sleep duration relied on few questions with multiple adjustments [4]. Hence, we put forth an effort of estimating the impact of total sleep deprivation on lipid parameters as if sleep can inoculate humans from CVD.

Insulin like growth factor – 1 (IGF-1) is one of the principal somatomedins. Secretion of IGF-1 is independent of growth hormone (GH) before birth but it is stimulated by GH after birth, and it has pronounced growth promoting activity [5]. Hence IGF-1 is used as a biomarker for disorders associated with abnormal GH secretion [6].

Low circulating levels of insulin-like growth factor 1 (IGF1) within the normal range have been associated with several cardiovascular risk factors, and hence play an important role in the development of cardiovascular diseases [7-9]. Though few studies have hypothesized the influence of shorter sleep duration on IGF-1 levels, the effect of total sleep deprivation is lacking. Hence, we aimed to determine whether total sleep deprivation alter the gene expression of IGF-1 and to examine the co-ordination of IGF-1 levels with serum lipid parameters among SD induced rats and compare it with the controls. *Withania somnifera* (WS) known as Ashwagandha is widely used in Ayurvedic system of medicine in India [10]. It possesses anti-inflammatory, antitumor, antistress, antioxidant, immunomodulatory, hemopoetic and rejuvenating properties [11]. Despite the availability of literature on the medicinal properties of WS and its chemical constituents, no reports exist on its hypocholesteremic effect in sleep deprivation in relation to the tropical growth factor IGF-1. We, therefore, have attempted to investigate the effect of WS on IGF-1 and lipid metabolism in total sleep deprivation induced rats.

Materials and Methods

Our study was conducted with the standard guidelines and protocols of Institutional Animal Ethics Committee (IAEC No: 007/2017). The rats obtained for our study were healthy adult wistar category weighing 120-150gm. The rats were sensitized in the animal house under laboratory conditions of temperature (22±21˚C), humidity (45±5%) and 12 h day: 12 h night cycle, with an access to food and water *ad libitum.* A total of 24 rats categorized into 4 groups (6 animals in each group) as Group I - cage control, Group II - large platform control, Group III - sleep deprived group & Group IV – WS treated SD group. Serum total cholesterol (TC), triglyceride (TG), low-density lipoproteins (LDL), and High-density lipoproteins (HDL) were assessed using assay kits purchased from spin react, Spain. Results are expressed as mg/dl. Gene expression and protein expression analysis of Insulin like growth factor by Real Time-PCR was done. Statistical analysis was done by one way ANOVA and Duncan's multiple range tests.

Sleep Deprivation Technique: Modified Multiple Platform Method

Total sleep deprivation was induced among rats for a period of one week by exposing them to modified multiple platform method. 8 circular platforms (7cm diameter) were mounted inside a customized water tank filled with water upto 1cm. The rats placed on the platforms when falls asleep, their muscle tone was lost and as a result they fall into the water tank. By climbing up back the rats were made to awake from sleep. The rats belong to large platform control group were placed on circular platforms with 14cm diameter. Hence even if the rats fall asleep, they slept on the platform as it remains large. In such way by keeping the rats in the same environment as like the sleep deprived group, it acts as a proper control group for sleep deprived rats [12].

Collection and Authentication of *Withania somnifera*

The plant was purchased from Tamilnadu Agricultural University, Coimbatore. Based upon the organoleptic and macroscopic examination the plant was authentically certified as *Withania Somnifera* (L.) Dunal of Solanaceae family by the Botanist, Prof. P. Jayaraman, Ph.D., Director, Institute of herbal botany, Plant Anatomy Research Center, West Tambaram, Chennai, Tamilnadu, India. The registration number of the certificate is PARC/2016/3232.

Ethanolic Extract of *Withania somnifera*

The roots of WS were air dried under shadow and powdered and the ethanolic extract was prepared with 95% ethanol for 12 hours in Soxhlet extractor. Then the extract was concentrated using rotary vaccum evaporator between 40°C to 60°C. The semisolid extract which is concentrated was stored in a refrigerator between 2-8°C. For experimentation purpose, the extract was dissolved in DMSO (Dimethyl sulphoxide) and administered orally to animals using a gastric lavage tube for 30 days with a dosage of 400mg/kg bw[13].

Assessment of Lipid Markers in the Serum of Control and Sleep Deprived Rats

Serum total cholesterol (TC), triglyceride (TG), low-density lipoproteins (LDL), and High-density lipoproteins (HDL) assessed using standard methods with assay kits purchased from spin react, Spain. Results are expressed as mg/dl.

Estimation of Insulin like Growth Factor-1

The mRNA expression of IGF-1 in the cortex of control and sleep deprived rats was done using real time PCR. Protein expression was done using western blot analysis.

Statistical Analysis

The data obtained were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the control and treatment groups using a computer-based software (Graph Pad Prism version 5). In Duncan's test, the significance was considered at the level of $p<0.05$.

Results

Each value represents Mean ± SEM of 6 animals. Significance at $p<0.05$, a-compared with control; b-compared with large platform control; c-compared with sleep deprived rat. A significantly increased level of total cholesterol, triglyceride (TG) and LDL cholesterol (LDLC) with decreased level of HDL cholesterol (HDLC) was observed in sleep deprived rats when compared to cage control rats and large platform control rats. Concurrently a significant rise in the serum concentrations of LDLC and TG were observed in LPC when compared to cage control rats which signifies the environmental influence on the rats. Though TC and HDLC does not show any changes in LPC rats. The rats who have undergone treatment with *W. somnifera* for a period of one month when deprived sleep showed significantly ($p<0.05$) reduced serum levels of TC, TG, LDL-c and significantly ($p<0.05$) increased HDL-c levels in contrast with SD rats without WS treatment (Table 1).

Table 1. Effect of *W. somnifera* on Lipid Profile Among Experimental Groups

Parameters	Normal control	Large platform control	Sleep Deprived rats	*W. somnifera* treated Sleep deprived rats	P Value
TC (mg/dl)	65± 5	70 ± 5	135 ± 5[a,b]	87 ± 2.5[a,b,c]	<0.0562
LDL-c (mg/dl)	52 ± 3.5	82 ± 3[a]	124 ± 6[a,b]	65 ±5[a,b,c]	<0.0014
TG (mg/dl)	85± 5	111 ± 9 [a]	139± 2.5[a,b]	95 ± 5[a,b,c]	<0.0014
HDL (mg/dl)	37 ± 2.5	36 ± 2.6	17 ± 3[a,b]	35 ± 1.8[a,b,c]	<0.0562

P value <0.05 statistically significant.

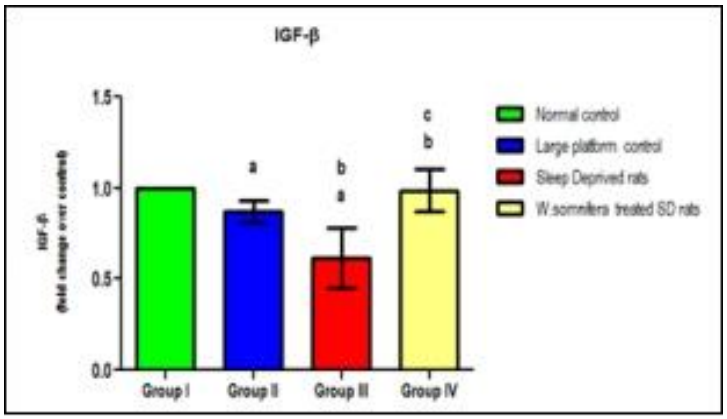

Figure 1. Effect of *W.somnifera* on IGF-1 mRNA Expression Among Experimental Groups

Each bar represents mean ± SEM of 3 observations representing 6 animals. Significance at p<0.05, a-compared with control; b-compared with large platform control; c-compared with sleep deprived rats. A significant negative correlation was found between the mRNA expression of IGF-1 and sleep deprived rats when compared with cage control and LPC groups. Simultaneously there was a significant down regulation of IGF-1 observed in LPC when compared to cage control rats. Pre-treatment with *W. somnifera* root extract (400 mg/kg b.wt) for about one month significantly up regulated the mRNA expression of IGF-1 in the rat's cerebral cortex (Figure 1). Hence the group treated with WS showed a positive correlation with mRNA expression of IGF-1.

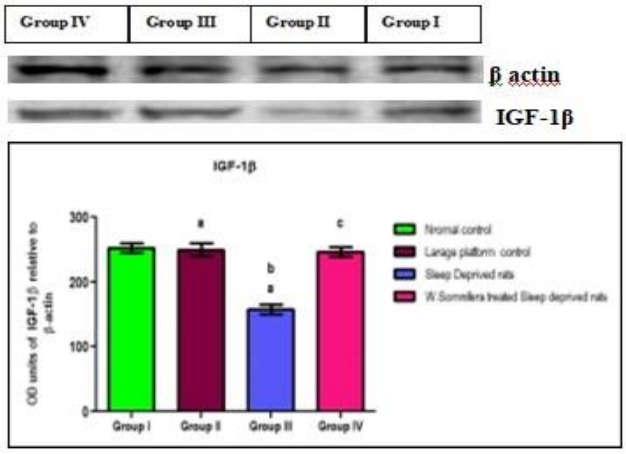

Figure 2. Effect of *W.somnifera* on the Protein Expression of IGF-1 Among Experimental Groups

Each bar represents mean ± SEM of 3 observations representing 6 animals Significance at $p<0.05$, a-compared with control; b-compared with large platform control; c-compared with sleep deprived rats. The protein expression IGF-1 was found to be significantly down regulated in sleep deprived rats when compared to control and LPC groups. Simultaneously there was a significant down regulation of IGF-1 in LPC when compared to control group. Pre treatment with *W. somnifera* root extract (400 mg/kg b.wt) significantly up regulated the mRNA expression of IGF-1 β in the cortex (Figure 2).

Discussion

In the present study, the biomarkers of lipid metabolism such as TC, TG & LDL-c were elevated in the serum of SD rats. In contrast to weight loss in animals, sleep deprivation induced hyperphagia which could contribute to the differences observed in the lipid parameters in our study. Along with this, the high TC, TG, LDL-c might also be due to stress (sleep deprivation) induced glycogenolysis by the hypothalamic–pituitary–adrenal (HPA) axis and secretion of stress hormones. Another possible mechanism might be due to the imbalance in the peptide hormones leptin and ghrelin as the regulatory activity of HPA axis is monitored by leptin sourcing from adipocytes. Increased food intake among sleep deprived rats might also be due to imbalance in the ratio of these hormones [14].

In addition, increased sympathetic activity in sleep deprivation stress could down regulate the leptin gene in the white adipose tissue which affects the integrity of HPA axis. Also evidence show that under conditions of negative energy balance, such as starvation, cachexia, and anorexia nervosa, ghrelin secretion increases. The above circumstances of imbalance among the hormones act on various components at different levels of the HPA axis and stimulate the paraventricular nucleus of hypothalamus to release corticotropin-releasing hormone (CRH). As a result, cortisol is released that elevates the body's blood-glucose level, which in turn creates more triglyceride production. This high triglycerides in turn could increase the cholesterol levels [15].

Anabolic activity of the GH/IGF-I axis regulates the body composition, bone density; muscle mass, as well as the development and maintenance of neuronal plasticity. Indeed, the growth-promoting effects of GH, at many tissue sites it is mediated partly by the GH stimulated IGF. The serum levels of IGF-I are modulated by different physiological (age, sex, nutrition, exercise, sleep, etc.) and pathological (illness, stress, etc.) conditions.

In our study the growth factor IGF-1 showed a significant down regulation in sleep deprived rats when compared to control rats. This might be due to the decline in the basal secretion of growth hormone following sleep deprivation which is an important regulator of IGF-1 secretion leading to a decreased hepatic production as well as circulating IGF-I levels.

As evidence, numerous studies have demonstrated that sleep, particularly SWS, increases the activity of the GH axis in normal subjects and the significant increase of IGF-I in blood with sleep extension could be induced by activation of its cellular gene expression and protein production in the liver. In our study we observed a decrease level of both mRNA and protein expression of IGF-1 in the brain cortex. Also, it stated the importance of IGF binding protein where he found that the decreased IGF-I in sleep deprivation may be influenced through basal GH and IGFBP-3 levels, the most abundant binding protein of IGF-I (i.e., 75% of the circulating IGF pool) which is considered as the major IGF-I carrier. Hence sleep deprivation is known to suppress circulating trophic factors such as insulin-like growth factor (IGF)-I [16, 17].

Various lipid metabolic pathways are influenced by IGF-I and GH. Both substances

have shown a noticeable ability to lower the total cholesterol levels in plasma, which could be of potential importance for clinical applications. IGF-I mimics insulin and stimulates glucose uptake and metabolism, probably acting through the insulin receptor. On the basis of the dual effector theory, concerning adipose tissue IGF-I would act on differentiated adipocytes with a limited capacity to proliferate [18, 19].

However, the way in which lipid metabolism is influenced by this growth factor especially in sleep deprivation is not well established. Hence our present study has investigated the influence of IGF-I on lipid metabolism in sleep deprivation through the determination of serum lipid levels as well as the analysis of the actions of *withania somnifera* and its interactions on the lipolytic activity of IGF-I.

Our results demonstrated that IGF-1 responded to sleep deprivation as protein expression of IGF-1 was found to be significantly down regulated in sleep deprived rats when compared to control and LPC groups. Simultaneously there was a significant down regulation of IGF-1 in LPC when compared to control group which again notably indicates the environmental influence. The down regulation of IGF-I in our study could reasonably raise the TC, TG &LDLc due to the inhibition of its hypo-cholesteremic effect on sleep deprived rats. In a study conducted by the phytochemicals present in the ethanol extracts of *Withania somnifera* root stimulated growth in fish species by dietary supplementation of it. These phytoconstituents in WS are reported to stimulate immunity, act as a substrate for various biochemical reactions, inhibitors of enzymatic reactions, enhance the absorption and provide stability of essential nutrients in the intestine. These are found to promote DNA, RNA, and protein synthesis and to stimulate GH and IGF1 production. Also, Withanolide A mediates lifespan extension and promotes stress resistance via insulin/insulin-like growth factor signalling pathway. So far, no study has reported the effect of *Withania somnifera* on IGF-1 secretion in sleep deprived rats. To our knowledge this is the first study investigating the effects of sleep deprivation on IGF -1 gene expression in relation to lipid metabolism including the role of *Withania somnifera* on it.

In the group treated with *Withania somnifera,* the levels of the growth factor IGF-1 were upregulated. The active components such as Withaferin A and Withanolide E normalized the levels of growth factor by regulating the growth hormone secretion. In addition, the reversal effect of *Withania somnifera* on lipid profile in our study might be due to the steroidal lactones (Withaferin A) and Withanolides in the root extract. The results obtained in this study therefore suggest that the hypocholesteremic effect of WS could be mediated through an increased bile acid synthesis for elimination of body cholesterol. The increased hepatic antioxidant activities in WS root powder indicate that fiber, phytosterols, polyphenols, flavonoids and vitamin-C in WS root could contribute to amelioration of the hyperlipidemic conditions [20]. Hence, our data suggest that *Withania somnifera* can minimize changes in the lipid profile parameters by up-regulating IGF-1 growth factor among sleep deprived rats. Hence our findings could be helpful to develop a therapeutic medicine for sleep related cardiovascular diseases from this natural compound.

Note: Large platform control is the actual control for the sleep deprived rats because in large platform the rats will be placed in the same environment (on large platform) as like the sleep deprived rats. But the animals are able to sleep since it is a large platform and won't fall down as in sleep deprived group (since it is a small platform). Cage control rats will be inside the cage and in a different environment. Hence large platform control

will be an accurate control group for sleep deprived rats.

Conclusion

In conclusion, the study demonstrated an inhibitory effect of sleep deprivation on IGF-I concomitant to increased serum TC, TG, LDLc & decreased HDLc levels. Therefore, it is possible that inhibition of the IGF-I system by sleep deprivation may provide a mechanistic basis for the hypercholesteremic changes on lipid metabolism. In addition, we observed changes in IGF-1, TC, TG, LDLc & HDLc which correlated positively with *Withania somnifera* treatment. With further research, these findings may prove to be important in maintaining proper sleep hygiene to limit disorders associated with high cholesterol and the significance of IGF-I in maintaining it.

Conflict of Interest

The author declares that there is no conflict of interest in the present study.

Acknowledgment

Nil.

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