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Withania somnifera Root Extract Enhances Telomerase Activity in the Human HeLa Cell Line

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Abstract

Aging is a decelerating unidirectional process of life. Shortening of telomeric DNA, the $(TTAGGG)_n$ hexanucleotide repeats, which form the caps at the chromosome ends, is implicated to determine the aging process, and more importantly the healthy lifespan itself. Telomerase, a ribonucleoprotein having reverse transcriptase activity, arrests telomere loss through addition of the TTAGGG repeats de novo, to the ends of the chromosome. The telomere/telomerase maintenance is an inevitable necessity to delay aging and for a healthy lifespan. Here, we report the potential of full-spectrum, high concentration Ashwagandha (*Withania somnifera*), an Ayurvedic medicinal herb, root extract to increase telomerase activity. HeLa cells, when treated with various concentrations of Ashwagandha root extract, showed an increase in telomerase activity measured with the established Telomerase Rapid Amplification Protocol (TRAP) assay. Ashwagandha root extract increased telomerase activity with highest enhancement of ~45% at 10 - 50 µg concentration. Thus, Ashwagandha root extract has the anti-aging inducing potential.

Keywords

Ashwagandha, Telomerase, HeLa Cell Line, TRAP Assay

1. Introduction

Aging, the relentless decelerating phenomenon, is the main cause for wide range of age-associated diseases like cancer, ischemic heart disease, stroke, type 2 diabetes, neurodegenerative diseases including Alzheimer's disease and others [1]. One of the major factors that accelerate aging and cause degeneration of various systems is

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the shortening of "TELOMERES", the DNA repeat sequences at the end of linear eukaryotic chromosomes, of 2 - 20 kbp length, which act as caps to conserve chromosome integrity and stability [2]. During the process of DNA replication, telomeres are shortened by 50 - 100 bp with each cell division. The end replication problem leads to critically short telomeres and ultimately senescence. Hence, telomeres are implicated as one of the factors that determine aging and lifespan. The enzyme, telomerase, a ribonucleoprotein having reverse transcriptase activity, carries out telomere replication. Telomerase synthesizes telomeric DNA sequences through the addition of TTAGGG repeats at the chromosome ends [3]. But, the telomerase activity is extremely low in somatic cells.

Telomerase is shown to be essential for the healthy normal life. Turning off of the telomerase expression in the conditional knockout mice leads to degeneration of multiple systems, including immune, digestive and nervous system. When telomerase expression is turned on later in life, these degenerations can be reversed. More importantly, nervous system, where neurons are thought to be differentiated and stay that way for a lifetime, manifests remarkable improvement from the degeneration [4]. Telomerase activity and short telomeres are implicated in aging mechanism [5]-[7]. Enhancing telomerase activity is one way to delay aging. A small molecule telomerase activator, TA-65, isolated from the root of the traditional Chinese medicine, *Astragalus membranaceus* in mice [8] can precisely do the same.

Ashwagandha (*Withania somnifera* Dunal) root extract, consisting of several withanolides, is an extensively used ayurvedic medicine with multitude of protective effects on humans such as revitalization, stress tolerance [9] and anti-inflammation [10]. Further, it enhances longevity in *C. elegans* [11]. With little or no toxic effect, Ashwagandha [12] can be considered as supplement in humans [13]. Though Ashwagandha is suggested to increase longevity, so far no evidence has been provided to reinforce this claim except in *C. elegans* [11] which is usually a harbinger to the positive outcome in humans.

Here, we address the efficacy of a high concentration, full-spectrum Ashwagandha root extract to provide anti-aging effect through enhanced telomerase activity in an *in vitro* cell culture model.

2. Materials and Methods

2.1. Cell Culture

The human HeLa cell line was used in the present study. The cells were maintained in DMEM (Invitrogen) supplemented with 10% fetal bovine serum in a humidified incubator (37˚C and 5% CO_2). Cells (40% - 60% confluency) were treated with various concentrations (10 µg, 50 µg, 100 µg, 500 µg, 5 mg) of Ashwagandha Root Extract (KSM-66, Shri Kartikeya Pharma, Hyderabad) for 72 hours.

2.2. Preparation of Cell Lysate

Cells were collected and centrifuged at 6000 rpm for 6 min. Pellets of 6×10^5 cells were suspended in ice-cold NP-40 lysis buffer and incubated 30 min on ice [14]. The cell lysate was aliquoted into separate tubes and stored at −80˚C until analyzed by TRAP assay.

2.3. Telomerase Assay and Quantification

Detection of telomerase activity in HeLa extracts employed the PCR-based telomeric repeat amplification protocol (TRAP assay) is a modification of the assay described by [14]. The protein concentration was determined using Lowry's method [15]. Briefly, total volume of reaction mixture was 50 µl contained 0.5 µl of dNTP mix (2.5 mM), 5 µl of 10× TRAP Buffer, 0.15 µg TS [5'-AATCCGTCGAGCAGAGTT-3'] primer, 0.15 µg and ACX [5'GCGCGGCTTACCCTTACCCTTACCCTAACC-3'] primer, 40 µl RNase-free H2O, 0.5 µl of 5 U/µl Taq DNA polymerase and 2 µl (1000 cells) of cell lysate. The PCR mixture was incubated at 30 min at room temperature. The PCR was then started at 94˚C for 90 sec followed by a 40-cycle amplification (94˚C for 20 s, 50˚C for 30 s, and 72˚C for 90s). The amplified PCR product will yield a 6-bp incremental ladder was separated on 12% acrylamide-TBE gels and visualized by ethidium bromide staining in UV light. The images were then processed and quantified using Image J software. The density/intensity of the amplified 6 bp repeat periodicity PCR product observed and quantitated by Image J software is represented as densitometric units.

2.4. Statistical Analysis

Statistical analyses on the percentage of telomerase activity in HeLa cell extracts were done using Sigmaplot

(ver.10.0).

3. Results

Aging associated telomere shortening is well documented. More importantly, telomerase, which is needed to maintain the telomere length through replication, is essential for healthy life. In the terminally differentiated somatic tissues, telomerase activity is low. Several factors, including stress aggravate the senescence process through telomere shortening. Given the importance of telomere/telomerase in aging and health, identification of a supplement from the natural/plant herb based ancient traditional systems of medicine, which could retain the telomere length or increase telomerase activity, is a less expensive and faster way with the most important added benefit of dismal toxicity. We evaluated the Ashwagandha root extract powder (KSM-66) suspended in water to enhance telomerase activity. Using the human cervical carcinoma cell line, HeLa, reported to have telomerase activity [16], we determined Ashwagandha's telomerase activity enhancing effect following published protocols [14]. First of all, we determined the optimal total protein concentration of the cell extract needed to assess the telomerase activity (**Figure 1**). The telomerase activity was highest between 50 ng and 5 µg of total protein of cell extract. We selected 2 µg as the ideal protein concentration and all further telomerase assays were carried out under these conditions.

3.1. Identification of Optimal Concentration of Ashwagandha to Enhance Telomerase Activity

HeLa cells were exposed to different concentrations of full spectrum Ashwagandha root extract as suspension of the powder in water for 72 hrs following which the cells were processed and telomerase activity determined. This resulted in dose-dependent increase in telomerase activity upto 50 µg /ml after which the activity started decreasing as the preparation is a powder suspension (**Figure 2**).

3.2. Enhancement of Telomerase Activity

Further, HeLa cells were treated with 10 µg and 50 µg of Ashwagandha root extract based on results arrived from dose response curve. Cells treated with 10 - 50 µg of Ashwagandha root extract enhanced telomerase activity by 45% (**Figure 3**).

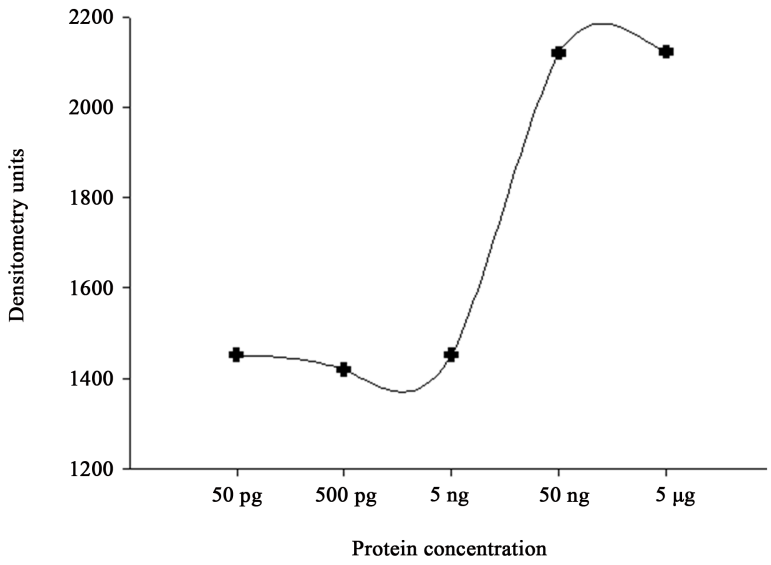

Figure 1. Determination of optimal concentration for the telomerase assay. Different concentrations of HeLa cell lysates were assayed for telomerase activity. The activity is measured by quantifying the density of amplified 6 bp repeats under each concentration with Image J software and represented as densitometric units in the Y-axis. X-axis represents the amount of total HeLa cell lysate as protein concentration.

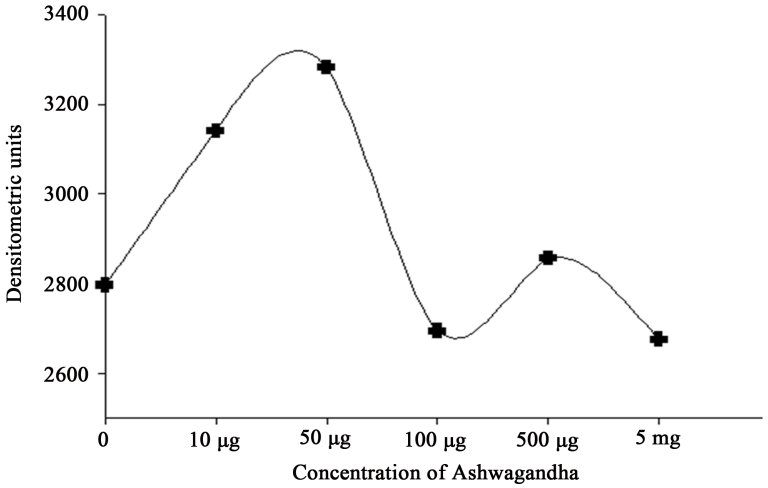

Figure 2. Effect of various concentrations of Ashwagandha root extract on telomerase activity in HeLa cells. The concentrations of Ashwagandh is given in the X-axis. The telomerase activity measured by quantifying the density of amplified 6 bp repeats (resolved in the 12% TBE-acrylamide gel), under each concentration with Image J software and represented as densitometric units in the Y-axis.

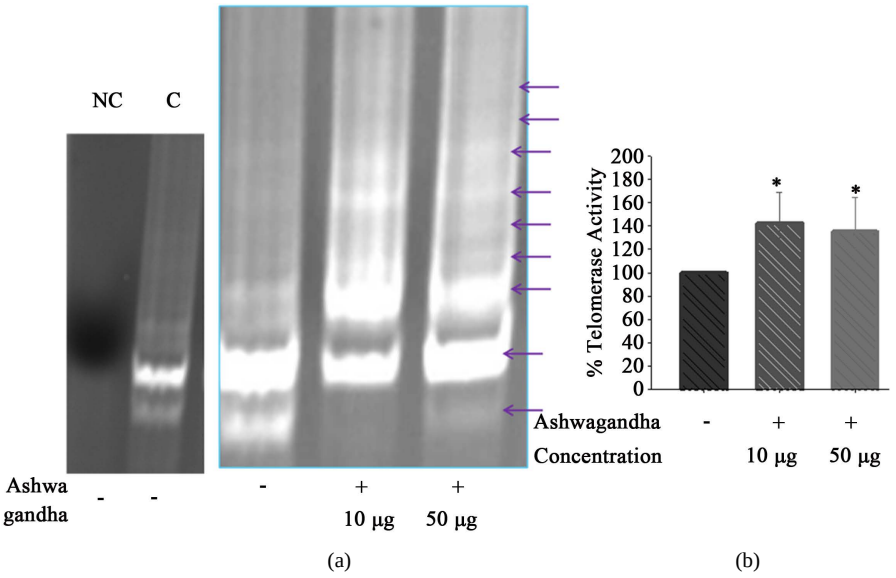

Figure 3. Results of Quantitative TRAP assays: (a) Gel picture showing 6-bp ladder of telomerase activity resolved in 12% TBE-polyacrylamide gels. NC-Negative control—without Hela cell lysate; C—with HeLa cell lysate; with and without Ashwgandha treatment; The arrows represent the amplified 6-bp periodicity products due to telomerase activity followed by PCR amplification; (b) Densitometry analyses of percentage of telomerase activity in control and ashwagandha treated cells (Cumulative of four independent sets). Statistical significance $^{*}P < 0.05$.

4. Discussion

As non communicable diseases are in the rise due to aging, strategies to increase healthspan is rigorously evaluated using all possible systems and organisms. Generally, a gene or its absence or specific chemicals like Reserpine [17] [18], Aswagandha [11], Rapamycin [19] and Resveratrol [20] can increase lifespan in various organisms like yeast, *C. elegans*, drosophila and mice. In addition, they induce stress tolerance. But the exact mechanism of their lifespan extension, especially the contribution of telomere/telomerase is not known. One of the

important qualities for health span is stable telomere at the ends of the chromosomes in the differentiated somatic tissues. The hallmark of aging is associated with telomere; the progressive attrition of telomere in human beings contributes to mortality in several age-related diseases [6] [21]. Mouse cells without telomerase activity have short telomere length [22], emphasizing the essentiality of telomerase for telomere maintenance. Since aging promotes degenerative pathologies, screening for new drugs is a necessity to develop highly effective therapy for the enhancement of telomerase activity in order to avoid critically short telomere length leading to aging and age-related diseases. Tomás-Loba *et al*., (2008) [23] demonstrated that enhanced telomerase activity in mice can delay aging and confer cancer resistance. Recently, Bernardes de Jesus *et al*., (2011), demonstrated TA-65 [8], the telomerase activator obtained from the Chinese medicine, can elongate short telomeres and increase health span of mice without increasing cancer incidence. Hence, we evaluated the enhancement of the telomerase activity, which is to maintain the telomere by adding the hexanucleotide repeats, by an ancient Ayurvedic herb Ashwagandha root extract (**Figure 1**). Indeed, Ashwagandha root extract powder, at a concentration of 10 µg - 50 µg/ml (**Figure 2**), increased telomerase activity by ~45%, in the Human HeLa cell line upon 72 hrs exposure (**Figure 3**), as determined by the TRAP assay. Ashwagandha is one of the most widely used ancient ayurvedic herb and generally non-toxic. Moreover, earlier, we reported that Ashwagandha can increase *C. elegans* lifespan [11]. Therefore, Ashwagandha deserves to be evaluated as a potential anti-aging ayurvedic herbal preparation in higher organisms and the potential mechanism needs to be investigated.

5. Conclusion

Thus, Ashwagandha root extract is able to enhance telomerase activity in the HeLa cell line. Given the exceptional necessity of the telomeres at the ends of the chromosomes for the maintenance and integrity of the chromosomes, it will be worthwhile to evaluate Ashwagandha under various adult onset disease conditions. This can provide a holistic protective effect.

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