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Influence of *Amalaki Rasayana* on telomerase activity and telomere length in human blood mononuclear cells

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ABSTRACT

Background: Indian traditional medicine practices use defined *rasayana* preparations to improve the quality of health in aged individuals. *Amalaki Rasayana* is one such *rasayana* prepared from the fruits of *Phyllanthus emblica* and is popularly used to prevent or treat various age related health conditions. Telomerase activity in the cells maintains telomere length and is implicated in ageing and various diseases wherein the shortening of telomere during ageing is controlled chiefly by the telomerase activity.
Objective: In the present study, we investigated telomerase activity and telomere length in the peripheral blood mononuclear cells of aged individuals administered with *Amalaki Rasayana*.
Materials and methods: *Amalaki Rasayana* was administered to healthy, aged (45–60 years) volunteers for 45 days after *koshta shuddhi* procedure. The telomerase activity and telomere length were analyzed on 0, 45th and 90th days of *Amalaki Rasayana* administration in peripheral blood mononuclear cells from these individuals and compared with age-matched placebo group and young volunteers (22–30 years). The data were compared between the groups.
Results: The results indicated an increase in telomerase activity with no discernible change in telomere length in the *Amalaki* administered participants. The comparison between young and aged participants revealed higher telomerase activity in young participants with no significant differences in telomere length.
Conclusion: The data indicate that the maintenance of telomere length is facilitated by an increase in telomerase activity upon *rasayana* administration in aged individuals and *Amalaki Rasayana* may prevent the erosion of telomeres over a period of time in aged individuals to promote healthy ageing.

1. Introduction

Ageing is a multifactorial, irreversible phenomenon regulated by intrinsic and extrinsic factors. These play a major role in imparting heterogeneity during ageing process and the longevity among the species may rely on their genetic makeup and the environmental factors. The intricate process of ageing is explained not only as a decline of functions but also as the one that involves complex multifactorial mechanisms [1,2]. The functional decline due to ageing is broadly classified as a) programmed theories in which ageing depends on biological clocks and b) error theories in which sustained and progressive accumulation of DNA damage, free radicals and macromolecular cross-linking that occurs due to environmental effects [2]. Ageing is also reported due to senescence associated with shortening of telomere length (replicative senescence) or cellular stress (cellular senescence) [2].

Telomere is a specialized nucleoprotein structure that determines the terminal segments of linear chromosomes consisting of tandem repeats of DNA sequences characterized by clusters of G residues [3] and it is necessary for maintaining chromosomal

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stability by allowing the complete replication of the 5′ ends of the chromosomal DNA. Telomeres are regularly shortened at each cell division [4], resulting in replicative senescence. The broken ends of chromosomes lacking telomeres are also subjected to recombination or terminal degradation leading to progressive loss of internal sequences. However, the maintenance of telomere length is regulated by telomere specific telomerase enzyme [5,6], which prevents cellular senescence [7]. Ageing is associated with the decrease in telomerase activity [8,9] and telomere length [10,11]. Although telomerase activity is often undetectable in several human cells, the germ line cells [12], cancer cells [13] and actively dividing peripheral blood mononuclear cells [14], show detectable levels and it varies with age [9].

Rasayana is one of the branches of Ayurveda-based traditional medicinal system, which deals with the rejuvenation, regeneration, immunomodulation and healthy ageing [15]. Many fruits, herbs and spices are blended in precise proportions to prepare *rasayanas* of various types and traditionally used to promote health. According to *Charaka Samhita*, the administration of *rasayana* increases the longevity of life, memory, comprehension, health, youthfulness, brightness and complexion [16]. *Amalaki Rasayana* which is prepared from fruits of *Amalaki* (*amla*; Indian gooseberry, *Phyllanthus emblica*) is widely used in the Indian traditional system of medicine as a cardiac, cerebral and intestinal tonic [17]. *Amalaki Rasayana* is grouped under *Vayasthapana rasayana,* which is reported to promote longevity, prevent ill health and block geriatric symptoms. *P. emblica* is a good source of ellagic acid, gallic acid, quercetin, kaempferol, emblicanin, flavonoids, glycosides proanthocyanidins and vitamin C [18] and has been studied to overcome several human ailments due to its reported chemical compositions [17,18]. The vitamin C, tannins, alkaloids, phenolic compounds and flavonoids present in *amla* also possess immunomodulatory, antioxidant and anticancer activities [18,19].

Considering an imbalance in telomerase activity and telomere length as an important hallmark of ageing and that *Amalaki Rasayana* consists of variety of reported properties of an anti-ageing herbal preparation, in the present study we designed to identify the influence of *Amalaki Rasayana* on telomere length and telomerase activity in aged human participants.

2. Materials and methods

2.1. Rasayana preparation

Amalaki Rasayana was prepared at Arya Vaidya Sala, Kottakkal by following standard procedure as per Ayurveda texts [16]. In brief, the dry gooseberries were pulverized and then mixed with freshly extracted gooseberry juice prior to drying. The dry mass was then pulverized and again blended with juice. These steps of pulverization, blending and drying were repeated 21 times. The final *Amalaki* powder was blended with ghee and honey in the ratio of 2:1:4 parts to obtain a thick pasty mass of *Amalaki Rasayana* (Fig. 1). The placebo was also prepared by Arya Vaidya Sala, Kottakkal, which contained wheat powder, honey and ghee. These were packed in small containers and the net weight of each *Amalaki Rasayana* and placebo was 45 g.

2.2. Selection of participants and administration of Amalaki Rasayana

According to Ayurveda, the age group of 45–60 years is considered as the age of onset of geriatric symptoms. Therefore a total of 116 healthy, non-smoking, non-alcoholic males between the ages of 45–60 years were selected for this study by Ayurvedic doctors (Vaidya) at the SDM College of Ayurveda, Udupi. The study was approved by the Institutional Ethics Committee and all experiments were performed in accordance with the guidelines of the ethics committee. Consenting participants who matched the inclusion criteria underwent general physical check-up by trained physician at Kasturba Medical College, Manipal. The participants with chronic and acute disorders were excluded. Those selected for the study were coded and underwent *Koshta Shuddhi* (body purification) procedure for 6 days. *Koshta Shuddhi* includes two days of *snehana* (oleation), two days of *abhyanga* and *bashpa swedha* (fomentation or sudation), one day of *virechana* (purgation) and two days of *samsarjana* (normalization of diet). *Amalaki Rasayana* or placebo was given a day after *samsarjana* (7th day onwards). The duration of the *Amalaki Rasayana* or placebo administration was for 45 days. It was given as a single dose (45 g/day) at early morning on empty stomach. Young individuals (n = 51) between 22 and 30 years age group were also included to compare the differences between young and aged.

2.3. Sample collection

Blood samples from the aged participants were collected before *Koshta Shuddhi* (on initial day), after 45 days of *rasayana*/placebo administration (6 days of *Koshta shuddhi* and 45 days of *rasayana*/placebo administration) and 45 days after the completion of *rasayana*/placebo administration (90th day). The blood samples from the young participants were collected only once. The peripheral blood mononuclear cells (PBMCs) from 10 ml of blood were isolated by employing standard Ficoll Paque (GE Healthcare, Sweden) method. These cells were processed for telomerase activity and telomere length.

2.4. Protein extraction and telomerase activity

Protein was extracted from the samples and positive control (MCF-7; human breast cancer cells) using the NP-40 lysis buffer (0.1 ml of 1 M Tris–HCl, 0.125 ml of 0.02 M Sodium deoxycholate, 1.5 ml of 1 M NaCl, 0.1 ml of 0.5 M β-mercaptoethanol, 0.01 ml of 0.1 M 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, 0.01 ml of 1 M $MgCl_2$ and 8.15 ml of MilliQ water). In brief, about

Sl. No	Ingredients	Quantity
1	Amalaki (*Phyllanthus emblica* Linn.)	2 part
2	Madhu (Honey)	4 part
3	Ghrita (Ghee)	1 part

Fig. 1. *Amalaki Rasayana* and its ingredients.

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