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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 life 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

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residues [3] and it is necessary for maintaining chromosomal 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,

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.

0.01 ml of 1 M $MgCl_2$ and 8.15 ml of MilliQ water). In brief, about 80−90% confluent positive control cells were washed twice with ice-cold PBS. The cells were scraped and transferred into Falcon tubes, mixed and counted. Cells were pelleted by centrifugation (3000×g for 5 min at 4 °C) and suspended in NP-40 lysis buffer at the concentration of 1250 cells/μl. Cells were incubated for 30 min on ice and then snap frozen using liquid nitrogen for 10 min. These were then stored at −80 °C. Protein concentration was estimated by Bradford method [20].

The telomerase activity in PBMCs was analyzed using the previously reported procedure [21]. The telomeric repeat amplification protocol (TRAP) is a PCR-based method which involves two reverse primers (ACX:5′-GCGCGGCTTACCCTTACCCTTACCC-TAACC-3′; 1000 ng/μl and NT: 5′-ATCGCTTCTCGGCCTTTT-3′; 1000 ng/μl) and one forward primer (fluorescent labeled: Cy5-TS: Cy^5-5′-AATCCGTCGAGCAGAGTT-3′; 100 ng/μl). An oligonucleotide, TSNT (5′-AATCCGTCGAGCAGAGTTAAAAGGCCGAGAAGCGAT-3′; 1×10^{-18} mol/μl) was used as an internal control. TRAP master mix for each reaction contained TRAP buffer (0.4 ml of 1 M Tris−HCl; 1.26 ml of 1 M KCl; 0.16 ml of 0.125 M EGTA; 0.03 ml of 1 M $MgCl_2$; 0.01 ml of Tween 20 and 0.14 ml of MilliQ water), dNTP mix, TRAP primer mix (ACX and NT-1000 ng/μl; Cy5-TS − 100 ng/μl; TSNT − 1×10^{-18} mol), Cy5-TS (100 ng/μl), BSA (50 mg/ml), RNase free water (39.95 μl), *Taq* DNA polymerase (2 U) and protein extraction equivalent to 2000 cells. The reaction mixtures were incubated at 28 °C for 30 min for the extension of the substrate by telomerase. The extension products were amplified by PCR using the following PCR conditions: 95 °C for 5 min to inactivate telomerase, 95 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s, 72 °C for 3 min for final elongation. After TRAP reaction, about 25 μl of each sample was separated on a 10% non-denaturing acrylamide gel for 1 h at 250 V. The gel was fixed by incubating in 0.5 M NaCl, 50% (vol/vol) ethanol and 40 mM sodium acetate (pH 4.2) for 15 min. It was then visualized using a Phosphorimager (Fujifilm-FLA 5000: at 635 nm) and intensity of the telomerase products (6-bp ladder) and the 36-bp Internal control (IC) (TSNT primer band) was measured. The relative telomerase activity was calculated using the following formula: Relative Telomerase activity (RTA) = Intensity of Sample's TRAP ladder − Intensity of lysis buffer/Intensity of sample's IC band. It was normalized by using the formula: RTA after normalization = (RTA of the samples/RTA of positive control) * 100.

2.5. Telomere length analysis

Telomere length of the DNA from PBMCs of both young and aged human participants were analyzed using quantitative-PCR method [22]. In brief, genomic DNA was extracted from the samples by standard phenol-chloroform procedures and stored in 1X TE buffer at 4 °C. Samples from 95 aged (48 *rasayana* and 47 placebo) and 48 young human participants were analyzed for telomere length measurement by singleplex QPCR. The two sets of primers (Telomere primers-Telg and Telc; Single copy genes − AlbU and AlbD) were used for telomere length analysis of the samples. DNA from HeLa cells served as reference. The SYBR green based analysis of the Ct values of telomere (T) and single copy gene (S) of experimental DNA samples were carried out separately using 7500 Fast Real time PCR (Applied Biosystems, Foster City, CA). The ratio obtained by the Ct value of Telomere and single copy gene (T/S) was considered as the relative telomere length of the sample. The aged (21) and young (3) samples of recruited participants, which yielded poor quality DNA and protein on isolation and storage afterward were excluded from the study.

Statistical analysis: Student's t test was carried out using SPSS software to analyze the results of telomere length and telomerase activity in analyzed samples. The data are expressed as mean ± SEM. Differences were considered as statistically significant at $p < 0.05$.

3. Results

We analyzed PBMCs from 51 young and 95 aged (49 *rasayana* and 46 placebo administered) participants and MCF-7 breast cancer cell line (positive control) for telomerase activity. The kinetics of telomerase activity was assessed by using protein from different number of MCF-7 cells [Fig. 2]. The results showed significant increase ($p < 0.001$) of telomerase activity with increasing number of cells from 2500 to 10,000 cells [Fig. 2]. For each assay, proteins equivalent to 2000 cells were used based on the results of kinetic studies. The relative telomerase activity (RTA) was calculated for the test samples and the positive control. The RTA values of the test samples were then normalized by RTA value of the positive control.

The results indicated that MCF-7 cells showed high telomerase activity (27.35 ± 0.28) [data not shown]. The cells from young participants showed 10.3 ± 0.81 of normalized mean RTA value [Fig. 3]. Further, the normalized mean RTA value in young individuals was significantly ($p < 0.05$) higher when compared to that of aged participants (4.46 ± 0.24) [Fig. 3a]. The mean RTA of *Amalaki Rasayana* administered individuals at initial day, 45th day and 90th day were 4.48 ± 0.40, 4.53 ± 0.31 and 5.69 ± 0.43 of the positive control respectively. Similarly, the mean RTA of aged individuals administered with placebo samples analyzed at different time points were 4.43 ± 0.26, 4.72 ± 0.32, and 3.98 ± 0.28 of the control [Fig. 3b]. Therefore, the data of *rasayana* and placebo administered to aged individuals showed no significant difference in the telomerase activity on initial day and 45th day. However,
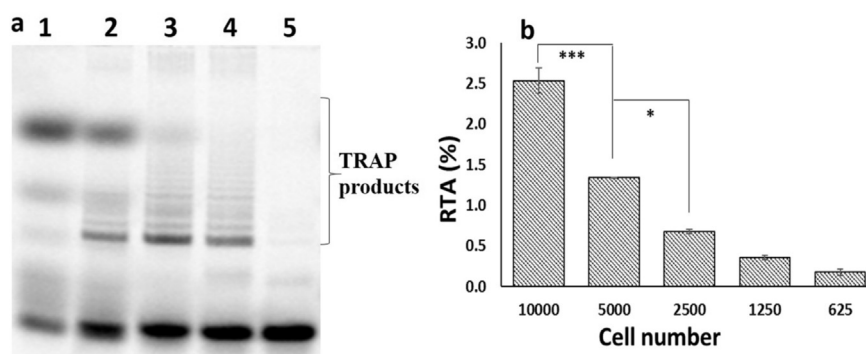

Fig. 2. Kinetics of telomerase activity in MCF-7 cells a) Gel picture showing telomerase activity when 10,000 cells (Lane 1), 5000 cells (Lane 2), 2500 cells (Lane 3), 1250 cells (Lane 4) and 625 cells (Lane 5) are used. b) Telomerase activity is increasing with increase of number of cells. The results are expressed as mean ± SEM.
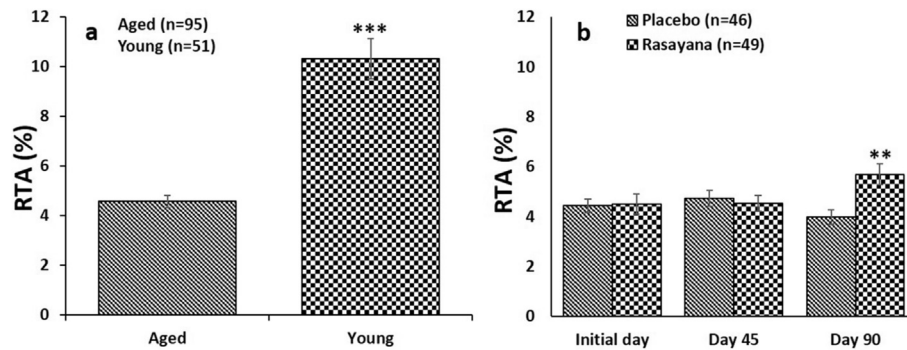
Fig. 3. (a) Normalized telomerase activity in aged (45—59 years) and young (22—30 years) individuals. (b) Telomerase activity in *rasayana* or placebo administered individuals at initial, 45th and 90th days. The results are expressed as mean ± SEM.

rasayana administered group of aged individuals showed higher level of telomerase activity at 90th day when compared to placebo individuals [Fig. 3b].

To correlate the effect of *rasayana* with respect to age, we subdivided the aged individuals into two groups of 45—52 years and 53—59 years respectively. The results showed a significant increase ($p < 0.05$) in the telomerase activity on 90th day in 45—52 years age group of individuals administered with *rasayana* (5.49 ± 0.55) when compared to respective placebo group (3.92 ± 0.38) [Fig. 4a]. Further, an insignificant increase in telomerase activity was observed in 53—59 years age group administered with *rasayana* [Fig. 4b]. On the other hand, a decreasing trend in RTA was observed in both age groups of individuals administered with placebo individuals on 90th day [Fig. 4].

A comparison of telomerase activity with respect to BMI showed no significant difference between normal (18—24 kg/m^2) and overweight (24—30 kg/m^2) individuals in both young and old volunteers. A comparison of telomerase activity with respect to BMI between young (normal −10.36 ± 0.89; overweight − 12.23 ± 3.59) and aged (normal − 4.40 ± 0.28; overweight − 4.41 ± 0.39) indicated higher telomerase activity in young individuals than the aged participants. Further, there were no significant variations in RTA between normal and overweight young or aged participants [Fig. 5].

Preliminary correlation of telomerase activity showed insignificant differences between normal BMI (3.91 ± 0.34, 4.35 ± 0.40 and 5.35 ± 0.66) and overweight (5.07 ± 0.72, 5.06 ± 0.51 and 5.58 ± 0.52) BMI groups administered with *Amalaki Rasayana* at initial, 45th and 90th day time points tested. However, *Amalaki Rasayana* administration showed pronounced increase in telomerase activity in normal BMI individuals when compared to overweight BMI individuals from 0 day to 90th day although it was statistically insignificant. The telomerase activity on the other hand reduced insignificantly from 0 day to 90th day in placebo administered individuals [Fig. 6].

Efforts were made to understand the effect of *Amalaki Rasayana* on telomere length in aged participants. DNA from HeLa cells was chosen as reference DNA [23]. The T/S ratio of HeLa cells was ~1 which was taken as internal standard. These cells were used as they show very high telomerase activity and the telomere length remains constant up to 23—26 cell doublings [23,24]. The comparison of relative telomere length between young and aged participants showed no significant differences [Fig. 7a]. Similarly, no significant differences were noticed when the aged participants of *Amalaki Rasayana* group in comparison with placebo group [Fig. 7b]. Classification of participants based on BMI or age also showed no differences (data not shown). Thus the overall data indicated there was no significant change in the telomere length by *Amalaki Rasayana* within 90 days of regimen.

Health indicators such as blood parameters were also analyzed at initial, 45th and 90th days of administration of *Amalaki Rasayana*. The results showed that the blood cell counts (white blood cells and subsets such as lymphocytes, monocytes, neutrophils, eosinophils, basophils and red blood cells and platelets), hemoglobin level, hematocrit (HCT), erythrocyte sedimentation rate (ESR), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV) and red cell distribution width (RDW) were within the reference range. The results also indicated no significant difference in the values of these parameters between selected young and aged volunteers. *Amalaki Rasayana* administration did not elicit changes in the values at 45th or 90th days when compared to initial day of treatment regimen.
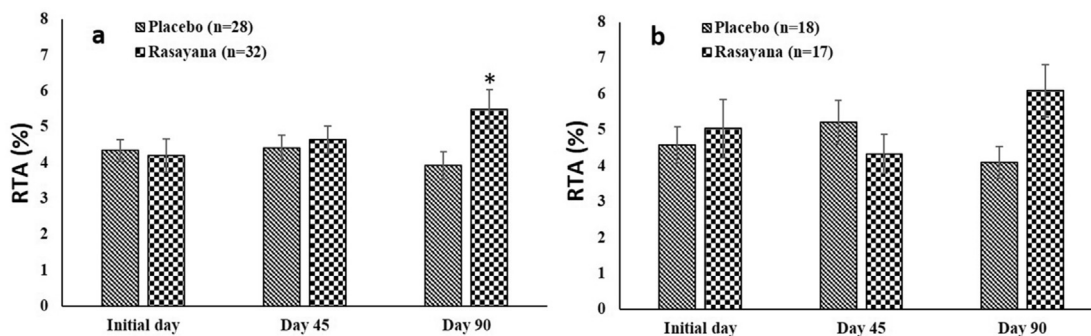

Fig. 4. Telomerase activity in *rasayana* or placebo administered individuals belonging to different age groups such as a) 45—52 years b) 53—59 years. The results are expressed as mean ± SEM.
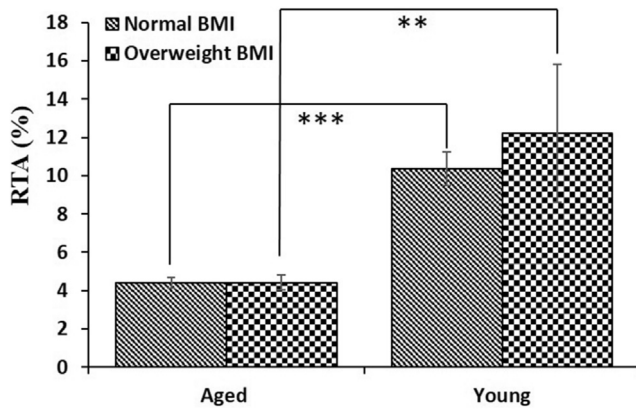
Fig. 5. Telomerase activity in young and aged individuals with normal BMI or overweight BMI. The results are expressed as mean ± SEM.

Further, no significant differences were observed between *rasayana* and placebo groups. The data suggests that the critical parameters that maintains health are unaltered. In addition, there were no adverse effects of *Amalaki Rasayana* on aged volunteers.

4. Discussion

Ageing is a complex biological process and recent evidences suggest that the ageing occurs rapidly in humans. Cellular senescence induced by lack of telomerase activity and shortening of telomeres is one of the postulated theories of ageing. Zhang et al. [25], have shown that inhibition of telomerase activity by doxorubicin in dose and time dependent manner correlated to growth of human hepatoma cells with shortened mean telomere length

indicating telomerase activity is important for cell survival. Correlation of telomerase activity with the proliferative capacity of the cells was also observed by Funakoshi et al. [13], in different types of tumors and normal cells. Benign and malignant tumor cells showed high telomerase activity compared to the normal cells. Fu et al. [26], using pheochromocytoma cells showed that telomerase suppressed apoptotic signaling cascades and cellular senescence, though it promoted tumor formation. These evidences clearly implicate telomerase and telomeres in cellular senescence and ageing. In the present study, MCF-7 cancer cell line showed high telomerase activity which was consistent with earlier report [20] and was thus used as positive control to understand the telomerase activity in young and aged participants. The present results suggest that the telomerase activity in peripheral blood mononuclear cells from young participants were significantly higher than in aged participants [Fig. 2]. Previously, a decrease in telomerase activity with an increasing age has been demonstrated [9] in two independent cultures of human amniotic fluid (AL1 and AL2). Telomerase activity in these cells at the first stage was 34% (in AL1) and 48% (in AL2) to that of HeLa cell line. The activity progressively decreased to 2.9 and 2.27% in the second stage, to 0.86% and 1.66% in the final stage in AL1 and AL2 cells respectively. Iwama et al. [8], also showed a decrease in the relative telomerase activities in PBMCs with increasing age.

Our results did not show significant differences in the telomere length between young and aged participants as well as between *rasayana* and placebo groups of aged individuals [Fig. 7]. This is consistent with the findings of Son et al. [10] who showed the progressive loss of telomeres in T and B lymphocytes from human blood was high in the newborns up to 4 years of age, with gradual decrease in the rate between age 4 and 39 years. Telomere shortening
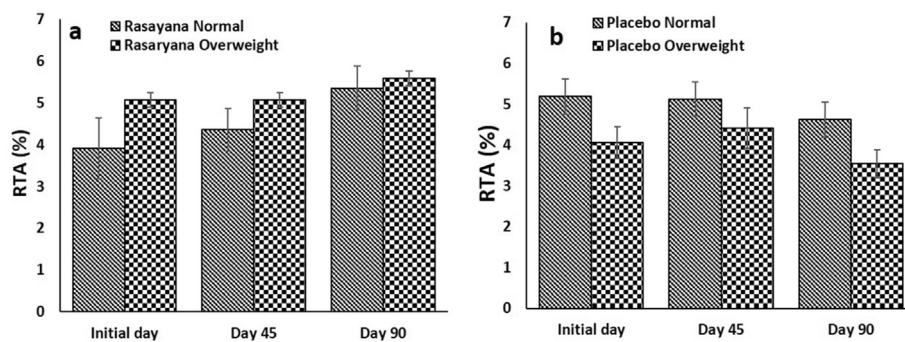

Fig. 6. Telomerase activity in a) *Amalaki Rasayana* administered b) Placebo administered aged individuals with normal BMI or overweight BMI. The results are expressed as mean ± SEM.
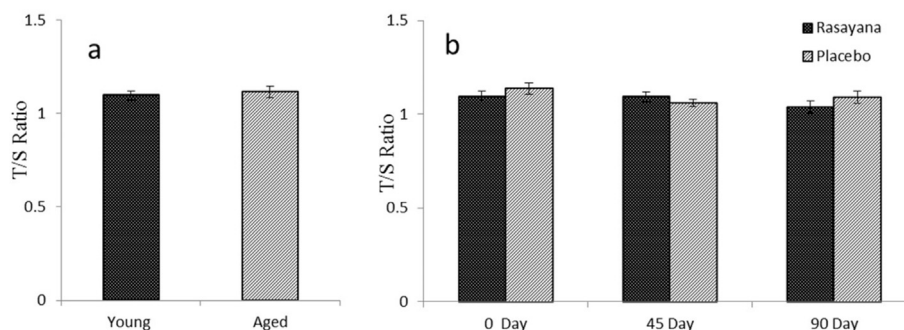

Fig. 7. Relative telomere length expressed as T/S ratio: a) between young and aged human participants and b) between *Amalaki Rasayana* and placebo administered aged individuals at initial, 45th and 90th days. The results are expressed as mean ± SEM.

continued at a relatively stable pace and was low between 40 and 95 years of age and these were dependent on telomerase activities.

The traditional medicines especially *rasayana* holds promise to improve the quality of ageing. *Amalaki Rasayana* is extensively reported to influence various biological activities to promote longevity and prevent geriatric symptoms. It is assumed that the health of the tissues in the body (*dhatus*) is influenced by the qualities of the *rasa-dhatu*. *Rasayanas* are such formulations suggested to improve the quality of *rasa* and hence helpful in strengthening or promoting the health tissues in the body [27]. *Amla* is the most commonly mentioned herb in the *Charaka Samhita* and suggested as good rejuvenating herb. *Amalaki Rasayana* was found to promote longevity, prevent ill health, block geriatric symptoms and increase fertility and personal appeal [28]. Ingredients of *Amalaki* are considered as *Vayasthapanum* (preserving of life), which suggests life supporting activities of *Amalaki Rasayana* [28]. In Ayurveda, it is described to possess appetizing (*Deepaniya*) and digestant (*Pachniya*) properties. As *rasayana*, *Amalaki* was also found to be effective on general health, age related changes, voice, sensory organs, physical capacity, psychological improvement and complexion [28]. Bargale et al. [29], showed that administration of *Amalaki Rasayana* with milk to be significantly effective against the symptoms such as insomnia, constipation, digestive weakness and hemoglobin percentage. Dwivedi et al. [30], have used the similar *Amalaki Rasayana* formulation at the concentration of 0.5% as food supplement and showed an increase in fecundity, size of salivary gland with additional cycles of DNA endo-replication, thermo tolerance, starvation tolerance, median life span, and shortening of developmental time. In the present study, we did not observe any ill effects in the individuals who are administered with *Amalaki Rasayana*. In addition to this, there was no change in the health of the individuals administered with *Amalaki Rasayana* as analyzed by the blood parameters at all the time points (Table 1).

Amalaki (*P. emblica*) is also included as one of the important ingredients in various Ayurveda herbal formulations, found to possess chondroprotective [31], anti-clastogenic [32], anti-inflammatory, hypocholesteromic, hepatoprotective, antioxidant [28] and antimicrobial [33] activities. Thus, it has several mechanisms of action and hence in the present investigations we focused on telomerase activity and telomere length which may influence cellular senescence and ageing. Cawthon et al. [34], while working on telomere length in human blood cells showed that there

was loss of about 14 base pairs of telomere sequence per year. The gradual loss of telomere during ageing leads to replicative senescence and apoptosis. Further, the mortality rate of the individuals over 60 years of age with shorter telomeres was twice than that of those with longer telomeres [34]. The median survival associated with shorter telomeres was 4–4.8 years [34]. Furthermore, the mortality rate from heart disease was five times and by infectious disease was eight times higher in the individuals with shorter telomeres [34]. Evidences also suggest that the higher telomerase activities found to increase the proliferation of cells [13,25] and suppress cellular senescence [26] in addition to elongation of telomere length. Delivery of mRNA encoding TERT to human fibroblasts and myoblasts found to increase telomerase activity which rapidly extends telomeres thereby increasing the proliferative capacity [35]. Thus assessing telomerase activity with respect to short time period during ageing is essential. Hence, in the present investigation, the telomerase activity was analyzed in aged participants by classifying them into two subgroups. The results have indicated that there was a significant increase in the telomerase activity in PBMCs of individuals in the *Amalaki Rasayana* group of aged participants at 90th day when compared to placebo-control participants (Fig. 3). Classification of individuals into two subgroups showed significant increase of telomerase activity in 45–52 years age group on 90th day. Insignificant increase in the telomerase activity was also noticed in 53–59 years age group (Fig. 4). This may be due to the action of phytochemicals of *Amalaki Rasayana*. Wei et al. [36], while working on stem cell formation and tissue regeneration showed elevation of telomerase activity by vitamin C in human and minipig periodontal ligament stem cells. Ellagic acid, another major component of *Amalaki Rasayana*, is also known to induce expression of human telomerase reverse transcriptase $\alpha+$ $\beta+$ transcripts in estrogen positive MCF-7 breast cancer cells [37]. Bodnar et al. [7], have shown extension of life span of telomerase negative cells viz., retinal pigment epithelial cells and foreskin fibroblasts after transfection of telomerase gene. Jesus et al. [38], also showed that a small-molecule activator of telomerase (TA-65) purified from the root of *Astragalus membranaceus* found to increase telomerase reverse transcriptase levels in mouse thereby elongating critically shortened telomeres when given as a part of the diet. Further, in order to maintain adequate immunity over a life span, lymphocytes require indefinite proliferation capacity. Thus they in turn require telomerase activity to extend their life span. Rufer et al., [39] have shown that expression of hTERT

Table 1
Assessment of blood parameters in young and *rasayana* or placebo administered aged participants.

Blood parameters	Reference range	Initial day	Initial day		45 days		90 days	
		Young group	Placebo group	*Rasayana* group	Placebo group	Rasayana group	Placebo group	Rasayana group
Total. W.B.C	4000–11,000 cells/cu.mm	6838.00 ± 217.07	6427.66 ± 1430.97	6073.81 ± 1537.35	6627.65 ± 1431.58	6583.33 ± 1192.43	6442.55 ± 1409.24	6676.19 ± 1285.72
Lymphocytes	20.0–30.0%	35.32 ± 0.85	38.7 ± 7.62	35.38 ± 6.17	35.68 ± 5.4	32.71 ± 5.0	35.25 ± 6.11	33.58 ± 4.38
Monocytes	2–6%	7.85 ± 0.20	7.85 ± 1.66	6.98 ± 2.14	8.09 ± 1.15	7.74 ± 1.49	8.05 ± 1.36	7.87 ± 1.46
Neutrophils	50–70%	49.77 ± 0.83	47.5 ± 6.37	52.6 ± 6.88	51.52 ± 7.26	54.35 ± 7.31	50.53 ± 7.51	53.83 ± 6.53
Eosinophils	2–4%	6.18 ± 0.52	6.02 ± 3.64	4.9 ± 2.27	5.03 ± 2.19	4.42 ± 2.22	5.66 ± 3.7	4.65 ± 2.19
Basophils	Up to 1%	0.65 ± 0.05	0.58 ± 0.29	0.57 ± 0.23	0.57 ± 0.24	0.55 ± 0.28	0.7 ± 0.37	0.58 ± 0.24
Platelet count	1.5–4.0 lakhs/cu.mm	257,083.33 ± 6505.17	245,022.72 ± 48,202.81	242,880.95 ± 51,757.03	237,956.81 ± 65,134.12	259,023.8 ± 42,500.2	245,636.36 ± 42,468.64	245,904.76 ± 40,051.07
R.B.C. count	4.6–6.2 millions/cu.mm	5.06 ± 0.06	4.94 ± 0.33	4.95 ± 0.25	4.94 ± 0.24	4.94 ± 0.30	4.95 ± 0.28	4.95 ± 0.29
Hemoglobin	11.0–16.0 gm/dL	15.52 ± 0.13	14.54 ± 0.94	14.80 ± 0.81	14.78 ± 0.88	15.06 ± 0.82	15.16 ± 2.73	15.05 ± 0.79
ESR	Up to 15 mm/h	3.70 ± 0.37	7.3 ± 4.45	6.69 ± 4.68	8.2 ± 4.09	7.71 ± 3.64	8.78 ± 4.13	8.38 ± 4.52
HCT	37.0–54.0%	46.06 ± 0.38	43.6 ± 2.48	44.1 ± 2.45	43.77 ± 2.49	44.22 ± 2.64	43.72 ± 2.61	44.46 ± 2.38
MCH	27.0–34.0 pg/cell	29.58 ± 0.20	29.88 ± 1.13	29.76 ± 1.54	29.94 ± 1.24	30.52 ± 1.56	29.95 ± 1.42	30.27 ± 1.41
MCHC	32.0–36.0 g/dL	33.75 ± 0.08	33.51 ± 0.74	33.52 ± 0.63	33.64 ± 0.78	33.52 ± 3.06	33.72 ± 0.62	33.77 ± 0.53
MCV	80.0–100.0 fL	88.88 ± 0.64	88.85 ± 4.08	87.73 ± 9.16	89.16 ± 3.43	86.69 ± 12.57	89.02 ± 3.89	89.55 ± 3.97
RDW	11.0–16.0%	13.54 ± 0.10	13.27 ± 0.99	13.15 ± 1.22	13.76 ± 0.81	13.55 ± 0.64	13.6 ± 0.61	13.47 ± 0.67
A.E.C	40–440 cells/cu.mm	347.37 ± 19.86	278.0 ± 123.27	293.23 ± 136.61	245.0 ± 109.9	251.61 ± 120.75	315.0 ± 113.67	261.29 ± 140.65

found to extend the life span of human T lymphocytes. Hu et al., [40] also demonstrated the upregulation of telomerase activity in B lymphocytes in the germinal center which is required to maintain the telomere length during active proliferation stage. The reactivation of these to memory B cells found to reduce the telomerase level. Similarly Weng et al., [41] demonstrated the correlation of T cell proliferation with the induction of telomerase activity. Thus, the increased expression of telomerase enhances the cell proliferation and life span of cells by suppressing senescence [42].

There are reports to show the relevance of BMI on health, quality of life, and ageing [43–45]. Furthermore, telomerase activity is thought to relate to body mass [44]. Hence, the classification of data was carried out based on BMI and we showed an insignificant increase of telomerase activity in normal BMI group of individuals from 0 to 90th day when compared to overweight individuals. On the other hand, the telomerase activities in placebo group of individuals were reduced insignificantly from 0 to 90th day (Fig. 6). In agreement with this, Seluanov et al. [46] showed significant negative correlation of telomerase activity with the body mass in various tissues of rodents. Amalaki Rasayana is usually taken for longer period of time, often years and regular consumption can provide longevity [16]. Therefore any significant change that may alter telomerase activity due to Amalaki Rasayana individuals over a long period of time remains to be studied.

In the present investigations we did not observe any difference in the telomere length among various groups tested. This may be due to stable maintenance of telomere length in these aged participants. Pickett et al. [47], have demonstrated that increase of telomerase activity results in loss of elongated telomere products as t-circles in human stimulated lymphocytes. This may be due to excessive telomere elongation that leads to activation of XRCC3-dependent rapid telomere length control mechanisms which generates t-circles. The trimming of telomere is important for the maintenance of stable telomere length which may be individual-specific and tissue-specific. The event may be evoked when there was an increase beyond the threshold length that triggers a shortening event. The maintenance of telomere length also involves the expression of ku86 [48] and shortening is associated with organismal life span [49]. Furthermore, oxidative stress is also implicated in telomere length regulation and telomerase activity during ageing. Reducing the oxidative stress by supplementation of antioxidants may reduce ROS induced damages in telomeres. Liu et al. [50], have demonstrated that treatment of mice for 2 months with an antioxidant, N-acetyl-ʟ-cysteine found to increase telomerase activity. Amalaki Rasayana also known to possess antioxidant activities [18], which may be responsible for increase of telomerase activity and maintenance of telomere length. Further studies are warranted to explore specific effects on the mechanisms of telomere regulation and function.

Amalaki Rasayana is taken over a long period of time, often through years and this may bring about incremental changes in the telomerase activity and telomere length to maintain the quality of cells/tissues. To the best of our knowledge, this is the first report showing Indian traditional medicine to improve quality of ageing by modulating telomerase activity. A study involving larger sample size and other biochemical and molecular studies may be necessary to identify all the telomere related effects due to rasayana on ageing.

5. Conclusion

The overall preliminary results presented here indicate that the telomerase activity is higher in young participants when compared to aged participants. A latent increase in the telomerase activity was found in individuals belonging to rasayana group compared to respective placebo group. The results indicated no significant differences in the telomere length among all the groups of individuals studied. Our results suggest that Amalaki Rasayana may enhance the telomerase activity appropriately in aged individuals which may be associated with the other related biological effects to promote quality of health. The increase of telomerase activity may delay the onset of ageing process by marking critical upper limit of telomere length. Our results also showed that Amalaki Rasayana did not induce any ill effects in the aged individuals.

Conflict of interest

None declared.

Acknowledgements

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