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Rejuvenation through Regeneration: Scientific basis of the Polyherbal formulation "IMMURISE"

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Abstract
All tissues and organs undergo a progressive regenerative decline with aging. Besides changes in local microenvironment the other confounders may be the intrinsic cellular changes or extrinsic exposure to harmful biological or chemical agents with impaired repair mechanism. Therefore, it is important to address the factors which blunt the regenerative potential leading to terminal dysfunction and ways to rejuvenate by retarding aging process through chemoprevention using natural products. The current study was undertaken to assess the regenerative potential of a patented polyherbal formulation "IMMURISE". Direct human supplementation was done after careful examination of toxicity profile *in-vivo* in Rat model, Cs137 radionucleotide and heavy metal levels along with microbial load in the formulation and declared safe for human consumption. Regenerative potential was assessed by circulating telomerase activity and DNA damage was estimated by COMET assay. Long term supplementation in healthy and diseased group revealed elevated serum telomerase activity and improvement in DNA damage repair suggestive of rejuvenation through regeneration of local stem cells population in target organ leading to overall wellness and clinical improvement.

Keywords: IMMURISE, aging, regeneration, telomerase, rejuvenation, comet assay

Introduction
Rejuvenation in medical terminology refers to reversal of the aging process wherein aging is an accumulation of incremental damage to macromolecules, cells, tissues and organs in a living body. However, if any of that damaged tissue or organ can be repaired or recreated through the internal processes of a body or system is called regeneration [1].

In humans, ageing represents the accumulation of changes in a human being over time. However, the exact causes of ageing are still unknown. Current theories are assigned to the damage hypothesis, whereby the accumulation of internally or externally induced damage within cells such as DNA breaks, oxidation of bases or DNA telomere shortening may lead to ageing [1-3]. It seems difficult for biologist to comeup with single mechanistic model and a definitive solution to stop or at least retard aging. Exact role of telomere in aging is still not clearly understood except the fact that short telomeres triggers a biological process that causes dramatic change in normal cellular function leading to premature death or senescence. This shortening of telomerase can be prevented by activation of telomerase, an enzyme vital for regeneration and longevity [4, 5].

Telomeres are the specialized repetitive DNA sequences at the ends of the linear chromosomes, and associated proteins, that serve to maintain the integrity of the chromosomes. Telomerase is a ribonucleoprotein DNA polymerase complex that maintains telomere length [6, 7]. The absence of telomerase activity in most human somatic cells results in telomere shortening during aging [7-9]. Telomerase activity can be restored in human cells by hTERT gene transduction or potentially via drug therapy; such extended-lifespan cells could be useful in the forms of cell therapy for age-related diseases [10]. On the other hand, the absence of telomerase acts as a limitation on cancer growth unless telomerase becomes reactivated [11-15]. Besides this several phytopharmaceuticals either alone or in combination as pure or crude extract form have shown antioxidant, anti-aging or protective effect against senescence [16-22].

The Ayurvedic system of traditional medicine lay emphasis on modulation of immune responseto alleviate the disease pathogenesis and aging through "Rasayan". Dikshit *et al*. (2000) have reported immunomodulatory activity of one of such Rasayan "Immunocin" containing polyherbal formulation by stimulating cellular and humoral immune response [23].

In the present study was undertaken to assess the effect of IMMURISE supplementation on

circulating level of Telomerase activity and DNA repair efficiency in subjects receiving IMMURISE supplement for 90 days.

Material and Methods

Bio-analytical Testing

The polyherb formulation IMMURISE is composed of five herbs classified under GRAS (Generally recognised as safe) category. The formulation was analysed for radioactive content at Environmental assessment division, Bhabha Atomic Research Centre, Mumbai, INDIA. It was certified that the level of Cs-^{137} in the sample is less than the minimum detection limit of 0.6 Bq/kg and the tablets are free from man-made radioactive contamination. Therefore the formulation is fit for human consumption from the radiological point of view. Heavy metal content of the formulation was done by LCMS at SICART, Anand, Gujarat, INDIA following standard International guidelines (AHPA, 2009) [24] Microbial load testing was conducted at PERD Centre, Ahmedabad, Gujarat, INDIA and counts are within permissible limit as per WHO guideline (WHO, 2007) [25]. Based on above finding the formulation was found to be safe and hence proceeded for animal toxicity studies and human supplementation study.

Animal toxicity study

The objective of this study was to assess the acute toxicity of the herbal formulation Polyherb-Immurize, when administered as a single oral dose to Sprague-Dawley rats (Male and Female) at a dosage of 0.07(Low), 0.7(Mid) and 1.05 g/kg body weight (High). The studies were performed following the guidelines laid down in Schedule Y of Drugs and Cosmetics Act 1940 and rule1945 by the Drugs Controller of India (2013). The selected rats were kept for acclimatization with the surrounding environment for overnight prior to experimentation. The rats were grouped into six rats / group and three rats/cage. The rats were fed with unrestricted diet and water till 14 days. Animal experimentations were performed at PERD research centre in accordance with OECD guideline for toxicity testing of chemicals following Good Laboratory Practice (GLP) [26].

Human supplementation studies

The study involved estimation of Telomerase activation (Study-1) and DNA damage repair ability (Study-2) of a patented polyherbal formulation “IMMURISE” in human volunteers. Study-1 involved 22 healthy human volunteers with 10 in test group and 10 in placebo group including 5Females and 6 males between age group of 40-80 years in each group and had no major illness in recent past with normal liver and kidney function. Study-2 involved 14 subjects with diabetes (n=4), fatty liver (n=1), polycystic ovary (n=1), squamous cell carcinoma of buccal mucosa (n=1), active smokers (n=4) and healthy individuals (n=3). The study was conducted at the clinic of Dr. Akshay Sevak at Junagath, Gujarat, INDIA purely on volunteer basis without any commercial interest of participants. All the patients were counseled for the drug and its possible effects. The Acute Toxicity and Microbial Load count of the drug has already been tested and found to be within allowable limit. Informed consent was taken from each volunteer after careful explanation of supplementation protocol. The supplementation protocol was for 90 days three tablets (3600mg) twice daily. Blood sample was collected by vein puncture in fasting state at Day0 (before supplementation) and on day 91(after completion of 90 days supplementation) in each case. Four ml blood sample was collected using vacutainer device in plain for study-1 (Telomerase assay) and in EDTA for study-2 (COMET assay) at above schedule. Serum was separated after 30min of complete clotting of blood in plain vacutainer and stored at -20 ºC till further analysis. EDTA whole blood tube was processed for mononuclear cell isolation by density gradient centrifugation for COMET assay.

Serum telomerase activity was estimated using ELISA kit following manufacture's protocol (TE-ELISA kit, My Bio source, USA) and Comet assay was performed following modified protocol of Singh *et al*. (1988) [27]. These two tests were outsourced to Institute of Human Genetics, Ahmedabad, Gujarat, INDIA. (http:\\www.geneticcentre.org\).

Results

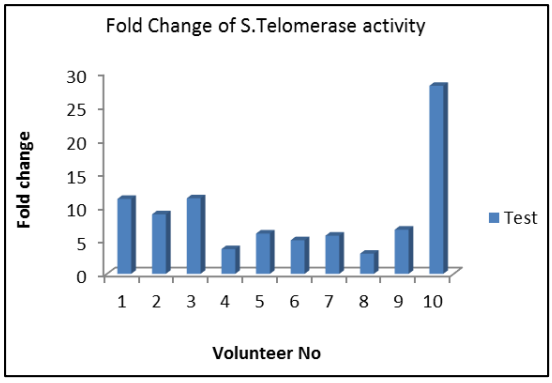
The result of heavy metal contamination as analysed by ICP revealed mercury and arsenic below detection limit and lead within acceptable limit as shown in table-1.

Heavy Metal Type	Total daily allowable conc	Conc in Immurise
Mercury	4-21 µg	BDL
Arsenic	≤20 µg	BDL
Lead	10-30µg µg	0.000124wt%

BDL: Below detection limit

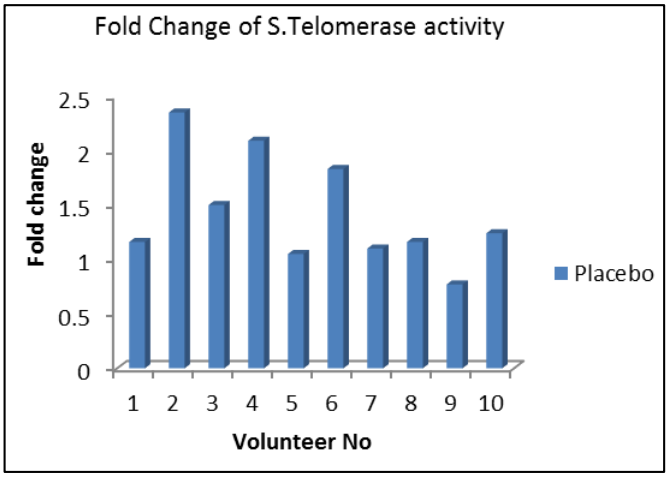
Fig.1 depicts S. telomerase activity in test and placebo group before and after supplementation of IMMURISE in study group-1. The fold change values, calculated as ratio of D90/D0, ranges from 0.77 to 2.35 with mean fold change of 1.43 in placebo group. However, in supplemented group the fold change ranged from 3 to 28 times with a mean fold change of 8.92. Result indicated a significantly high mean fold change in supplemented group as compared to placebo group (p=0.0194) which is suggestive of activation in telomerase activity due to supplementation.

In study group-2 comet assay was performed on WBC of test subjects which are healthy with or without tobacco habit and various chronic illness as indicated in Table-2. Result in Table-2 shows proportion of normal, damaged (mild/ moderate/high) and apoptotic cells in mononuclear cells of patients with various chronic clinical conditions before and after supplementation of IMMURISE in study group-2. Day 90 IMMURISE supplemented volunteer's WBC revealed significant rise in normal cell population with significant improvement in all the three damaged cell populations (mild, 0.0001; moderate, 0.0014; & high, 0.013 damage group) respectively with concomitant decrease in % of apoptotic cells (p-0.061; NS) suggestive of DNA repair activation possibly due to supplementation.



Supplemented (Test group)	*S. Telomease* Activity		Fold Change
	Before Supp	After Supp	
Mean	0.82	5.80	8.92
SE	0.25	1.75	2.69
Range			3-28
Paired "t" Test			P=0.0194

Placebo Group	S. Telomease Activity		Fold Change
	Before Supp	After Supp	
Mean	0.53	0.72	1.43
SE	0.16	0.22	0.43
Range			0.77-2.35
Paired "t" Test			NS



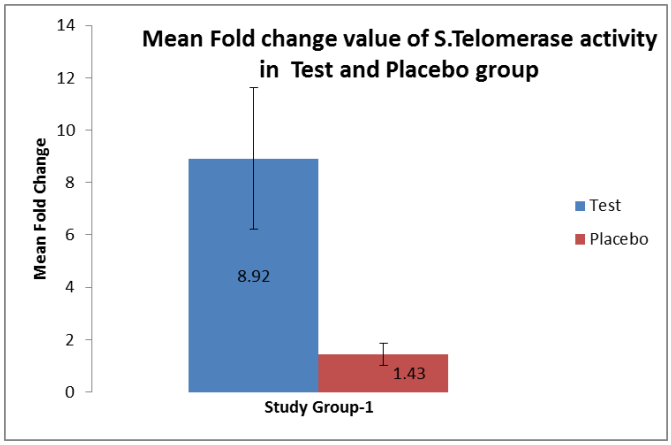


Fig 1(a, b, c): Individual and Mean fold change in S. telomerase activity in Test (n=10) and Placebo (n=10) group in study group-1

Table 2 (a, b): Proportion of normal, damaged (mild/moderate/high) and apoptotic cells in mononuclear cells of patients with various clinical condition before and after supplementation of IMMURISE in study group-2. (a)

Ref No	Normal (%)	Damage			Apoptosis (%)
		Mild (%)	Moderate (%)	High (%)	
CO-2A(43/M), Smoker	29.03	29.03	29.03	9.69	0.0
CO-3A(28/M), Smoker	39.21	35.29	21.56	3.92	0.0
CO-4A(28/M) Diabetic	40.0	31.42	17.14	8.57	2.85
CO-5A(70/M), Aged	37.14	31.42	17.14	14.28	0.0
CO-6A(65/F), Aged	16.66	30.0	26.66	26.66	0.0
CO-7A(41/M), Smoker	17.39	43.47	21.73	15.21	2.17
CO-8A(49/M), Smoker	68.75	16.25	10.0	1.25	3.75
CO-9A(40/F), SCC BM	63.75	26.25	2.5	1.25	6.25
CO-10A(38/M) Diabetes	64.63	30.48	2.43	1.21	1.21
CO-11A(54/M) Healthy	80.0	20.0	0.0	0.0	0.0
CO-12A(54/F) Diabetes	72.0	18.0	4.0	0.0	6.0
CO-29A (8/F); Juvenile Diab	17.7	13.7	3.9	45.1	19.6
CO-31A (58/F) Polycystic Ovary	40.0	36.0	14.0	4.0	6.0
CO-38A (35/M) Fatty Liver, NIDDM	20.0	11.0	22.0	25.0	22.0
Range	16.66-80.0	16.25-43.47	0-29.03	0-26.6	0-6.25
Mean	48.05	28.32	13.83	7.46	2.02
SEM	6..804	2.39	3.16	2.56	0.72

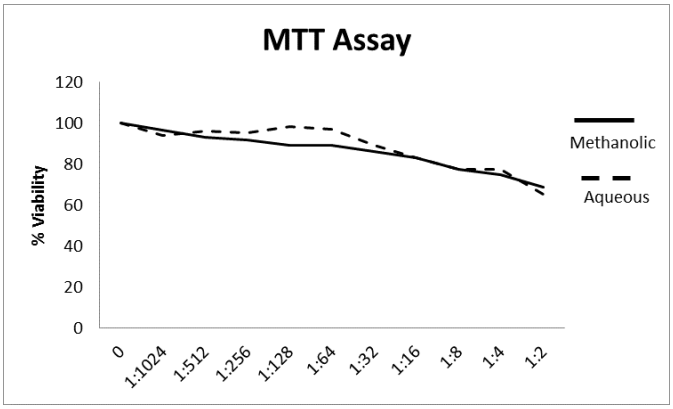
(b)

Ref No	Normal (%)	Damage			Apoptosis (%)
		Mild (%)	Moderate (%)	High (%)	
CO-2B	84.31	13.73	0.0	1.96	0.0
CO-3B	85.71	14.28	0.0	0.0	0.0
CO-4B	78.43	21.56	0.0	0.0	0.0
CO-5B	76.0	18.0	0.0	4.0	2.0
CO-6B	90.9	7.27	0.0	1.81	0.0
CO-7B	84.44	8.88	2.22	4.44	0.0
CO-8B	78.0	10.0	6.0	2.0	4.0
CO-9B	75.55	15.9	2.22	2.22	4.44
CO-10B	78.0	14.0	4.0	0.0	4.0
CO-11B	83.33	11.11	2.77	0.0	2.77
CO-12B	78.84	9.61	3.84	1.92	5.72
CO-29B	66.0	18.0	4.0	6.0	6.0
CO-31B	55.55	20.65	12.7	4.75	6.35
CO-38B	86.6	6.2	0	5.15	2.05
Range	75.5-90.9	7.27-21.56	0-6.0	0-4.44	0-5.72
Mean	81.23	13.12	1.91	1.66	2.08

SEM	1.45	1.29	0.63	0.475	0.66
Paired "t" test p-value	4.77; 0.0001	5.59; 0.0001	3.69; 0.0014	2.22; 0.038	0.061; 0.95 NS

NS: Not significant

MTT assay



Result of cytotoxicity assay on A549 cell line revealed least cytotoxicity even at highest concentration suggestive of no direct cytotoxicity of both aqueous and methanolic fraction. This result also supported nontoxicity of Immurise.

Discussion

Aging is a highly complex biological process made up of intricate interplay between cells and its microenvironment which are constantly changing and evolving. In spite of its complexity fundamental research on biology of ageing is advancing rapidly [28] DNA damage during the life span of an individual is an unavoidable event either due to oxidative stress from endogenous biochemical reactions generating reactive oxygen species or exposure to environmental mutagens leading to genotoxic injuries [29]. However, the stability of the genome is supported by an intricate machinery of repair, damage tolerance & prevention through free radical quenching that counteracts DNA damage [1, 2, 30, 31].

A lot of interventional studies have been done through supplementation of phytonutrients, immune modulators, hormone and growth factor substitute to increase the life span of laboratory animals, thereby achieving life extension [17-22]. A few experimental methods such as replacing hormones to youthful levels have had considerable success in partially rejuvenating laboratory animals and humans. Increased repair of damaged tissue and reversal of signs of aging have been reported by reactivation of telomerase enzyme [5]. There are at least eight important hormones that decline with age: 1. human growth hormone (HGH); 2. the sex hormones: testosterone or oestrogen/progesterone; 3. erythropoietin (EPO); 4. insulin; 5. DHEA; 6. melatonin; 7. thyroid; 8. pregnenolone. In theory, if all or some of these hormones are replaced, the body will respond to them as it did when it was younger, thus repairing and restoring many body functions [32] In line with this, recent experiments show that heterochronic parabiosis, i.e. connecting the circulatory systems of young and old animal, leads to the rejuvenation of the old animal, including restoration of proper stem cell function [33]. Similar experiments show that grafting old muscles into young hosts leads to their complete restoration, whereas grafting young muscles into old hosts does not. These experiments show that aging is mediated by systemic environment, rather than being an intrinsic cell property.

The DNA repair efficiency shows individual variability and hence variable susceptibility to the same carcinogen exposure. Therefore, cell fate is decided on the basis of the balance between extent of damage and efficiency of repair machinery [32, 34-36]. Time-dependent accumulation of damage in cells and organs is associated with gradual functional decline and aging. Ultimately it culminates in variety of cellular response e.g. transient G0 arrest of cell cycle, senescence induction or apoptosis. Cells undergoing apoptosis reveal a typical pattern of DNA fragmentation which can be assessed by simple comet assay to complex multi-caspase assays. The comet assay (single-cell gel electrophoresis, SCG or SCGE) is a well-established genotoxicity test for *in vitro* and *in vivo* testing of chemicals [35-38].

Piperakis *et al*. (2009) investigated DNA damage repair efficiency of freshly prepared lymphocytes and EBV transformed lymphocytes exposed to ionizing radiations at various passages by single cell gel electrophoresis or 'comet assay' [39] Older population showed higher base level DNA damage with declined repair efficiency. Earlier study by Gaivao *et al* (2009) have also reported a WBC based in-vitro model for estimating DNA damage and repair based on the comet assay, to measure the activities of repair enzymes in lymphocyte extracts prepared from repeat samples from normal, healthy subjects [39-42]. This justifies our study model involving COMET assay in subjects before and after supplementation of IMMURISE for 90 days. Decrease in tail length is an indicator of increased repair efficiency due to IMMURISE supplementation. A more recent study has demonstrated that mitochondria derived ROS play an important and direct role in the shortening of telomeres and the onset of senescence. Repair efficiency of telomeric DNA was reported to be lower in fibroblasts isolated from older human donors [43].

Telomerase is often mutated in human cancers and becomes constitutively activated and hence promote existing turnout growth faster [44]. In the race of telomere and telomerase a query always hunts our mind that whether activating telomerase activity will increase the risk of cancer. It is true that cancer cells express telomerase but not vice versa i.e. inducing telomerase expression in cells will make them cancerous [45]. To the best of our knowledge there are no experimental evidences in which activating the native telomerase has increased the risk of cancer. It has been reported earlier that transient induction of TERT by an astragalus-derived compound or using adeno-associated vims serotype 9 (AAV9)-based gene therapy in adult mice increases both health quality and life span without increasing cancer incidence [45]. It is true that telomerase does not catalyze the oncogenic process, however, it is equally true that it is required for the sustain growth of most advanced cancers. Two research groups one from Harvard lab of de Pinho and the other from a Spanish lab of Blasco focuses on the potential for telomerase to reduce cancer risk contradicting the assumption made one decade earlier [8, 14, 15]. DePinho argues that in normal cells telomerase should prevent neoplastic transformation by preventing DNA damage [12]. David Sinclair too agrees with the statement that activating telomerase might prevent turnouts and could restore organ function in the elderly and may be useful in

treating a variety of diseases related to aging [47]. Telomerase activation may possibly reduce the oncogenic transformation first by eliminating cells that are pro-inflammatory and potentially carcinogenic because their telomeres have become short, and secondly by rejuvenating the immune system, which is our primary defense against cancer.13 In 2007 under Patton Protocol-I, a natural product-derived telomerase activator supplement (TA-65, 10-5Omg daily) was provided with physician counseling/laboratory tests at baseline and every 3-6 months thereafter. The most striking observation was that the protocol lengthens critically short telomeres and replenish the relative proportions of circulating leukocytes toward the more "youthful' profile [48]. Replenishment of degenerating tissues with new proliferating cells can be achieved either by re-entry of senescent or quiescent somatic cells back into the cell cycle or via stem cell division. This may be feasible by reversing the damage signal imposed by p53 and its transcriptional target p21 due to damagerepair.[49]

Conclusion

In old age there is more to ageing than mere shortening of telomeres, however therapy which can activate telomerase could be combined with other therapies that target the biochemical pathways of ageing in order to extend healthy lifespan of human being. Current experimental findings including absence of radioactive molecules, absence of heavy metal and satisfactory in-vivo toxicity study ensures safety of IMMURISE for human consumption. Besides this the result of supplementation trial on healthy and diseased human subjects revealed increased telomerase activity in circulation and decreased genomic damage in MNC are suggestive of rejuvenating action of IMMURISE may be through metabolic activation of DNA repair enzymes and/or regeneration of stem cells in target organ leading to overall wellness. Further characterization of circulating secondary metabolites including growth factors, interleukins, cytokines and in-depth study of DNA repair pathway enzyme expression are required to decipher the currently unexplained role of IMMURISE.

References

1. Best BP. Nuclear DNA damage as a direct cause of aging. Rejuvenation Res. 2009; 12:199-208.
2. Hoeijmakers JH. DNA damage, aging, and cancer. N. Engl. J Med. 2009; 361:1475-1485.
3. Nakamura AJ, Redon CE, Bonner WM, Sedelnikova O. A. Telomere-dependent and telomere-independent origins of endogenous DNA damage in tumor cells. AGING 2009; 1:212-218 Jaskelioff, M. *et al*. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011; 469:102-7
4. Bodnar AG, *et al*. Extension of life-span by introduction of telomerase into normal human cells. Science. 1998; 279:349-352.
5. Jaskelioff M, *et al*. Telomerase reactivation reverses tissue degeneration in aged telomerase-deficient mice. Nature. 2011; 469:102-7
6. Ignacio Flores, Maria A Blasco. The role of telomeres and telomerase in stem cell aging. FEBS Letters. 2010; 584:3826-3830
7. Sadava D, Hillis D, Heller C, Berenbaum M. Life: The Science of biology. (9th ed.) Sunderland, MA; Sinauer Associates Inc, 2011.
8. de Jesus BB, Blasco MA. Telomerase at the intersection of cancer and aging. Trends Genet. 2013; 29(9):513-520
9. Hornsby PJ. Telomerase and the aging process. Exp Gerontol. 2007; 42(7):575-581
10. Ju Z, Lenhard Rudolph K. Telomere dysfunction and stem cell ageing. Biochimie. 2008; 90:24-32.
11. De Magalhaes JP, Toussaint O. Telomeres and telomerase: a modern fountain of youth? Rejuvenation Res. Summer. 2004; 7(2):126-33.
12. Artandi SE, DePinho RA. Telomeres and telomerase in cancer. Carcinogenesis. 2010; 31:9-18
13. Mitteldorf JJ. Telomere Biology: Cancer Firewall or Aging Clock? Biochemistry (Moscow). 2013; 78(9):1054-60.
14. Sahin E, Depinho RA. Linking functional decline of telomeres, mitochondria and stem cells during ageing. Nature. 2010; 464:520-528
15. Sharpless NE, DePinho RA. How stem cells age and why this makes us grow old. Nature Rev. Mol. Cell Biol. 2007; 8:703-713.
16. Anisimov VN, Poppvich IG, Zabezhinski MA. Methods of testing pharmacological drugs effects on aging and life span in mice. Methods Mol Biol. 2013; 1048:145-160.
17. Giovannelli L, Pitozzi V, Jacomelli M, Mulinacci N, Laurenzana A, Dolara P, *et al*. Protective effects of resveratrol against senescence-associated changes in cultured human fibroblasts. J Gerontol A boil Sci Med Sci.2011; 66(I):9-18.
18. Hao H, Nancai Y, Lei F, Wen S, Guofu H, Yanxia W, *et al*. Retracted : antiaging effects of purslane herb aqueous extracts and its mechanism of action phytother Res. 2009; 23(9):i-vii.
19. Obrenovich ME, Nair NG, Beyaz A, Aliev G, Reddy VP. The role of poluphenolic antioxidants in health, disease and aging. Rejuvenation Res. 2010; 13(6):631-43.
20. Russo A, Izzo AA, Cardile V, Borrelli F, Vanella A. Indian medicinal plants as antiradicals and DNA cleavage protectors. Phytomedicine. 2001; 8(2):125-32
21. Russo A, Izzo AA, Borrelli F, Renis M, Vanella A. Free radical scavenging capacity and protective effect of Bacopa monniera L. on DNA damage. Phytother Res. 2003; 17(8):870-75.
22. You J, Roh KB, Li Z, Liu G, Tang J, Shin S, *et al*. The Anti-aging properties of Andrographis paniculata by activation epidermal cell stemness. Molecules. 2015; 20(9):17557-69
23. Dikshit V, Kamre A, Kulkarni KR, Gokhla A, Saraf MN. Preliminary screening of IMMUNOCIN for immune-modulatory activity. IJPC. 2000; 62(4):257-60
24. Heavy Metals: Analysis and Limits in Herbal Dietary Supplements. American Herbal Products Association. Silver Spring, MD, December, 2009.
25. WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues. WHO, 2007
26. OECD guidelines for testing of chemicals. OECD/OCDE code 420, December 2001
27. Singh NP, MaCoy MT. A Simple Technique for quantification of low level of DNA damage in/individual cells. Exp Cell Res. 1988; 175:184-91
28. Gensler HL, Bernstein H. DNA damage as the primary cause of aging. Q Rev Biol, 1981; 56:279-303.
29. Hamilton ML, Van Remmen H, Drake JA, Yang H, Guo ZM, Kewitt K, *et al*. Does oxidative damage to DNA increase with age? Proc Natl Acad Sci USA. 2001; 98:10469-74.

30. Ames BN, Gold LS. Endogenous mutagens and the cause of aging and cancer Mutat Res 1991, 2503-16.
31. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A. 1993; 90(17):7915-22.
32. Urbanski HF, Sorwell KG, Garyfallou VT, Garten J, Weiss A, Renner L, *et al*. Androgen supplementation during aging: development of a physiologically appropriate protocol. Rejuvenation Res. 2014; 17(2):150-3.doi: 10.1089/rej.2013.1518. Epub 2014 Apr 8.
33. Conboy IM, Rando TA. Heterochronic parabiosis for the study of the effects of aging on stem cell and their niches. Cell cycle. 2012; 11(12):2260-67
34. Collins AR. The comet assay. Principles, applications, and limitations. Methods Mol Biol. 2002; 203:163-77.
35. Collins AR. The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol. 2004; 26(3):249-61.
36. Pazhanisamy SK. Stem cells, DNA damage, ageing and cancer. Hematol Oncol Stem Cell Ther. 2009; 2(3):375-84.
37. Liao W, McNutt MA, Zhu WG. The comet assay: a sensitive method for detecting DNA damage in individual cells. Methods. 2009; 48(1):46-53.
38. Lorenzo Y, Costa S, Collins AR, Azqueta A. The comet assay, DNA damage, DNA repair and cytotoxicity: hedgehogs are not always dead. Mutagenesis. 2013; 28(4):427-32.
39. Piperakis SM, Kontogianni K, Karanastasi G, Iakovidou-Kritsi Z, Piperakis MM. The use of comet assay in measuring DNA damage and repair efficiency in child, adult, and old age populations. Cell Biol Toxicol. 2009; 25(1):65-71. Epub 2007 Nov 24.
40. Gaivão I, Piasek A, Brevik A, Shaposhnikov S, Collins AR. Comet assay-based methods for measuring DNA repair *in vitro*; estimates of inter- and intra-individual variation. Cell Biol Toxicol. 2009; 25(1):45-52. Epub 2007, 5.
41. Fox JT, Sakamuru S, Huang R, Teneva N, Simmons SO, Xia M, *et al*. High-throughput genotoxicity assay identifies antioxidants as inducers of DNA damage response and cell death. Proc Natl Acad Sci U S A. 2012; 109(14):5423-8
42. Rundell MS, Wagner ED, Plewa MJ. The comet assay: genotoxic damage or nuclear fragmentation? Environ Mol Mutagen. 2003; 42(2):61-7.
43. Passos JF, Saretzki G, von Zglinicki T. DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Res. 2007; 35(22):7505-13. Epub 2007 Nov 5.
44. Burger AM, Fiebig HH, Kuettel MR, Lautenberger JA, Kung HF, Rhim JS. Effect of oncogene expression on telomerase activation and telomere length in human endothelial, fibroblast and prostate epithelial cells. Int J Oncol. 1998; 13(5):1043-8.
45. Shay JW, Wright WE. Role of telomeres and telomerase in cancer. Semin Cancer Biol. 2011; 21(6):349-53.
46. Mendelsohn AR, Larrick JW. Ectopic expression of telomerase safely increases health span and life span. Rejuvenation Res. 2012; 15(4):435-8.
47. Wu LE, Sinclair DA. Restoring stem cells - all you need is NAD. Cell Res. 2016; 26(9):971-2.
48. Harley CB, Liu W, Blasco M, Vera E, Andrews WH, Briggs LA, Raffaele JM. A natural product telomerase activator as part of a health maintenance program. Rejuvenation Res. 2011; 14(1):45-56.
49. Cox LS, Mason PA. Prospects for rejuvenation of aged tissue by telomerase reactivation. Rejuvenation Res. 2010; 13(6):749-54.