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Research Article

Assessment of Anti-aging Potential of a Poly-Herbomineral Blend: An *in Vitro* Study Lead

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Abstract

Aging is a natural biological phenomenon including oxidative stress, chronic inflammation, cellular senescence and cumulative metabolic dysfunction in human body. In Ayurveda 'aging', known as *Jara* - denotes natural aging process by progressive degradation of body tissues and declined in physiological function. Ayurvedic formulations are well known for their rejuvenation property and to support longevity, vitality and physiological balance. *DRDC/2015/003*, a Herbo-mineral blend derived by fortification of Chyawanprash with some well-reported Ayurvedic ingredients like Mukta (*Pearl calax*), Safed musli (*Chlorophylum borivilianum*) and Kesar (*Crocus sativa*) responsible for decreasing oxidative stress. Current *in vitro* study assessed anti-aging potential of *DRDC/2015/003,* on human fibroblast cell (HFF-1). The non-cytotoxic concentration of *DRDC/2015/003* was evaluated by MTT assay, followed by assessment of H_2O_2 induced beta galactosidase activity as oxidative stress marker. Results indicated to marked reduction (17.1% decrease) in H_2O_2 induced cellular senescence with β–galactosidase (SA- β-gal) activity, in the test setup as compared with controlled cells setup. Such observed activity of *DRDC/2015/003* in reducing H_2O_2 induced beta galactosidase activity in HFF-1 cells indicated to its significant role in anti- aging property and delaying premature cellular aging.

Keywords

DRDC/2015/003, Anti-aging, Antioxidant, SA β – Galactosidase, Cellular Senescence, Rasayan

1. Introduction

Aging is a process of metabolic alteration influenced by genetic, environmental, and lifestyle factors these are the increase oxidative stress largely mediated by hydrogen peroxide (H_2O_2) [1]. Persistent exposure of H_2O_2 disrupts cellular redox balance that cause DNA damage, mitochondrial dysfunction and activation of senescence-associated signalling pathway also drives the cell into senescence state where the cell stops dividing itself [2, 4]. It is known to release inflammatory molecules which led to tissue damage and induced cellular aging [3]. As per the Ancient Ayurvedic System of Medicine, Jara (aging) is caused due to imbalance of Doshas, tissue degradation and depletion of Ojas [4]. *DRDC/2015/003* is a Herbo-mineral formulation derived from the herbal blend of Chyawanprash which is consider as Rasayan in Ayurveda [5]. *DRDC/2015/003* combines traditional wisdom with modern sciences as in, it comprises ingredients well known for their Antioxidant and anti-aging properties. It has known benefits for maintaining overall heath, it also promotes vitality, vigour

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and youthfulness, which is a well reported characteristics of Chyawanprash [6, 7]. The potent ingredients of *DRDC/2015/003* are Amla (*Emblica officinalis*), Brahmi (*Bacopa monnieri*), Mukta (*Pearl calax*), Safed Musli (Chlorophylum *borivilianum*) and Kesar (*Crocus sativa*). All the ingredients are traditional rejuvenators. Mukta is commonly known as Moti in Ayurveda. It is helpful in nourishing *Ojas* (Vital essence of body) [8], Mushli supports Vigor and saffron possess neuroprotective [9] and mood – modulating benefits whereas Amla contains ascorbic acid and polyphenol which act as scavenger for free radical [10]. The studies have shown that that Mukta (pearl) one of the main ingredient of *DRDC/2015/003*, consist of protein (22.71 mg protein /g) and mineral content also it is enriched with essential amino acids such as Ldycine (Lys), Arginine (Arg), Histidine (His), Leucine (Leu), Phenylalanine (Phe) and Tryptophan (Try) [11]. The Protein, peptide and amino acids responsible for antioxidant potential which can be employed for treating various age-related degenerative disorders [12]. The aging process driven by the generation of Reactive Oxygen species (ROS), persistent oxidative damage signalling pathways that accelerates cellular aging [13]. In the present study we evaluated the protective response of *DRDC/2015/003* against oxidative stress-induce cellular aging in human diploid fibroblast cells (HFF-1). The premature senescence was detected in HFF-1 cells associated with H_2O_2 induced β-galactosidase activity where β-galactosidase serve as indicator of cellular senescence [14]. Studies demonstrate that in presence of Senescence-associated -beta galactosidase (SA β – gal), cells undergo irreversible cell- cycle arrest, thereby inhibited the duplication, due to oxidative stress. Hence, β – galactosidase activity was selected as biomarker of cellular senescence to assess the anti-aging potential of the *DRDC/2015/003*.

2. Material and Methods

Table 1. *Detail of DRDC/2015/003.*

Contents	Quantity (g)
Emblica officinalis	64
Mukta Pisti	0.028
Sesamum indicum seed oil	0.61
Clarified butter	1.22
Chlorophytum tuberosum, Mucuna prurita	0.1
Elttaria cardamomum	0.554
Syzygium aromaticum	0.062
Crystal Sugar	64
Honey	0.731
Aegla marmelos, Clerodendrum phlomidis, Oroxylum indicum, Stereospermum suaveolens, Gmelina arborea, Desmodium gangeticum, Urari picta,	0.285 each
Solanum indicum, Solanum surattence, Tribulus terrestris, Vitis vinifera, Tinospora cordifolia, Bacopa monnieri, Sida cordifolia, Inula racemosa	
Laptadenia reticulata, Phaseolus trilobus, Teramnus labialis, Pistacis integerrima, Phyllanthus niruri, Terminalia chebula,	
curcuma zedoaria, Cyperus rotundus, Boerhaavia diffusa, Nympaea stellate, Adhatoda vasica, Glycyrrhiza glabra, Martynia, annua,	
Cinnamomum zeylanicum, Cinnnamomum tamala, Mesua ferrea	0.107
Piper longum	0.258
Bambusa bambos	0.75
Abhrak Bhasma, *Anacyclus pyrethrum*	0.175
Chlorophytum tuberosum, Mucuna prurita	0.1
Crocus sativus	0.1

Contents	Quantity (g)
Syzygium aromaticum	0.062
Elettaria cardamomum,	0.554
*Each 100 g of Dabur DRDC/2015/003	

The cell culture grade chemicals and reagents were procured from Sigma-Aldrich, Bangalore, India. DMEM (Dulbecco's Modified Eagle's Medium wash GIBCO, and Cellular Senescence Activity Assay kit (Enzo life sciences) were used. Human fibroblast cell line i.e. HFF-1 was obtained from ATCC, USA. The *DRDC/2015/003* was obtained from Dabur India Limited, Ghaziabad, India. The Composition details of *Dabur's DRDC/2015/003* are given in Table 1.

2.1. Cell Line Maintenance and Sample Preparation

Human diploid fibroblasts cultured (HFF-1) cells were cultured in DMEM at 37°C, 5% CO_2 and 95% humidity. Subculturing of cells was carried out by trypsinization, subsequently transferred to fresh growth medium. *DRDC/2015/003* solution (200 mg/mL) were prepared in DMSO and L- Carnosine was used as positive control. All the experiments were performed in triplicates.

2.2. DRDC/2015/003 Effect on Cell Viability

The HFF-1 cells were seeded into 96 well plate (5 x 10^3 cells/well) with DMEM containing 15% FBS and incubated overnight in humidified chamber at 37°C & 5% CO_2. Then a range of *DRDC/2015/003* solution (1 µg/ml to 10,000 µg/ml/well) and L-Carnosine (125 to 500 mM/well) were added to HFF-1 cells and incubated for 96 hours. Spent medium was discarded and MTT solution was added and incubated for 3 hours. Followed by DMSO was added by pipetting out MTT solution to solubilize formazan crystals. Absorbance was measured at 540 nm using Synergy HT micro plate reader.

2.3. Cellular Senescence Assessment by β – Galactosidase Assay

HFF-1 cells were seeded (10 x10^3 cells/well) in DMEM (15%FBS) was incubated overnight, followed by addition of non-cytotoxic concentrations of DRDC/2015/003 and L-Carnosine. To understand aging effect, H_2O_2 (200µM/well) was added incubated for 2 hrs. Then the Senescence associated with β-galactosidase activity was assessed through kit (Enzo life sciences – ENZ- KIT129) following manufacturer instructions. The fluorescence was measured at 360nm / 465 nm (Biotek Synergy HT, Model No.) and% decrease in SA β –galactosidase activity was derived.3. Results

3.1. Cell Viability in Presence of DRDC/2015/003

The data of cell viability of HFF-1 cells was given in Table 2. DRDC/2015/003 has shown the cytotoxic activity towards HFF-1 cells at highest tested concentration i.e. 1000 µg/mL. Whereas HFF cells were viable in presence of *DRDC/2015/003* at 1 – 500 µg/mL, hence the SA-β-galactosidase activity was assessed non-cytotoxic concentrations. Similarly, L- Carnosine has shown cytotoxicity at 100 mM.

Table 2. *Percentage cytotoxicity in HFF-1 cells after 96 h of treatment*

Treatment	Test Concentration	% Cytotoxicity wrt untreated cells
RATNAPRASH SUGAR FREE (DRDC/2015/003) (µg/ml) in DMSO	0.1	7.52
	1	0.55
	15.6	2.57
	31.2	14.85
	62.5	1.25
	125	7.05
	250	2.73

Treatment	Test Concentration	% Cytotoxicity wrt untreated cells
L-Carnosine (mM) in DMEM	500	22.53
	1000	33.87
	12.5	18.55
	25	14.77
	50	22.53
	100	34.96

3.2. SA-β-Galactosidase Activity

The SA β – Galactosidase activity in presence of *DRDC/2015/003* and L-Carnosine was given in Figure 1. An inverse relationship was noted between%increase in β–Galactosidase activity and test compound i.e. DRDC/2015/003. Higher concentration of *DRDC/2015/003* i.e. 500 µg/ml have shown relatively less%increase of SA β – Galactosidase (Figure 1). While further lower concentrations of DRDC/2015/003 250 – 31.25 µg/ml have shown increased SA β – Galactosidase. Similar effect was noted with positive control i.e. L-Carnosine (Figure 1).

On the other hand, HFF-1 cells pre-treated with DRDC/2015/003 have shown dose dependent% decrease of SA β – Galactosidase in presence of H_2O_2 (Figure 1). However, the L-Carnosine pre-treatment has shown different pattern i.e. lowest test concentration (12.5µM) have shown highest% decrease of SA β – Galactosidase.

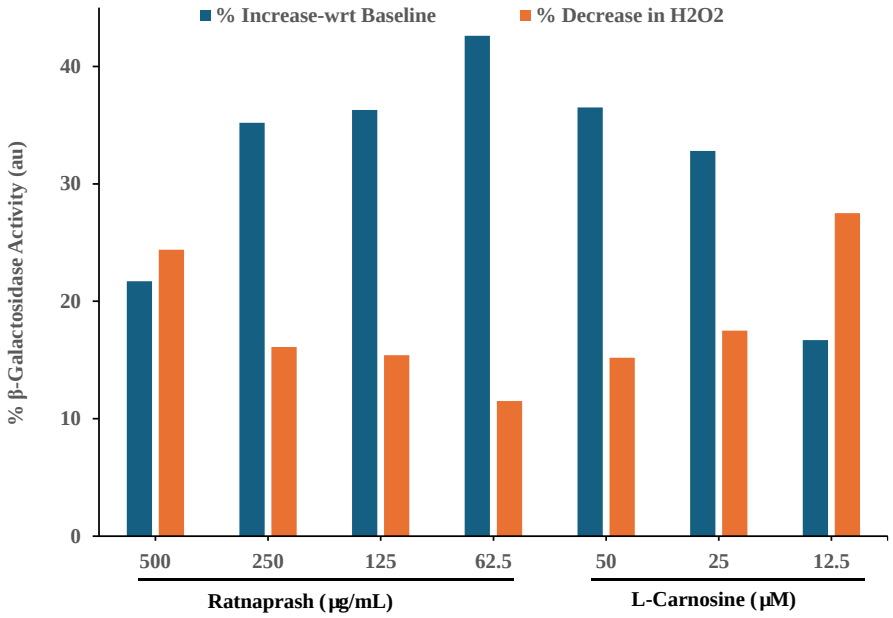

Figure 1. *HFF-1 Cell's β – Galactosidase Activity. Bars represent the% SA β – gal activity in presence of DRDC/2015/003 and L-Carnosine.% SA β – gal activity was increasing with reference to baseline with lesser concentrations of DRDC/2015/003, whereas, L-Carnosine has shown inhibitory action at lower concentrations compared to higher. Percent decrease% SA β – gal activity in H_2O_2 was decreased with lower concentrations of DRDC/2015/003, whereas L-Carnosine has shown inverse association.*

4. Discussion

Aging is the complex multi factorial biological process, cause due to oxidative stress, mitochondrial dysfunction and cellular senescence that eventually impairs cellular repair mechanism. As per Ayurveda aging (Jara) is natural yet changeable process causes due to depletion of Ojas and weakening of Dhatus [15]. This cause gradual decline in Physical strength, reduce vitality and impaired immunity therefore

Ayurveda emphasize Rasayan therapy well known for prolong vitality, vigour and rejuvenation effect [16]. *DRDC/2015/003* is based on classical Rasayan therapy principal. It consists of Herbal blend, supplemented with Mukta, saffron and Mushli antioxidant, antiaging, Geno protective and immunomodulatory potential [5]. Whereas Amal (*Emblica Officinalis*) contains antioxidant potential by reducing reactive oxygen species (ROS) and delay senescence [17].

Present study evaluated the antiaging potential and the effect of *DRDC/2015/003* on. The effect of *DRDC/2015/003* on SA β – galactosidase activity in stimulated HFF-1 (Human fibroblast) cell line. The cell line was pretreated with controlled sample of *DRDC/2015/003* (Concentration from 31.25 µg/ml to 500 µg/ml) for duration of 24 hrs subsequently challenged with 200 µM H_2O_2 to induce premature cellular aging. The cells were recovered from stress in presence of *DRDC/2015/003*. It led to 17.1% reduction in H_2O_2 induced beta galactosidase activity whereas DMSO led to 7.5% reduction in SA β – gal activity. This demonstrates the rejuvenative and antiaging potential of *DRDC/2015/003*.

Whereas L- carnosine was taken as positive control. It mainly acts as scavenger for reactive oxygen species (ROS). It also gives Antioxidant effect and reduces oxidative stress caused by H_2O_2. The cell line was pretreated with controlled sample of L- carnosine (Concentration from 12.5 µg/ml to 50 µg/ml) may reduce H_2O_2 induced beta galactosidase activity at all the concentration tested. According to study presented by G. A McFarland and R. Holliday had represented the DMEM supplemented with carnosine, it was found that 10 mM, 20mM, and 30 mM. A much greater reduction in growth rate was seen with 50 mM carnosine. This shows Dose- dependent effects of L- carnosine [13].

The present study provides comprehensive in-vitro analysis supports anti-aging Potential of *DRDC/2015/003*. This formulation demonstrated cellular compatibility with test concentration, confirming its safety for internal administration. When Hydrogen peroxide induces to normal fibroblast cells (HFF-1) it induces Oxidative stress (which is the mediator of senescence), it significantly increases in SA β – galactosidase activity. Subsequently, HFF-1 cells pretreated with *DRDC/2015/003* was able to overcome the Oxidative stress and led to reduction in SA β – galactose activity and improve cellular recovery. The anti-senescence effect of *DRDC/2015/003* associated with antioxidant activity and it contributes to neutralise reactive oxygen species and maintain homeostasis at cellular level. Nevertheless, the properties of *DRDC/2015/003* formulation as Anti- inflammatory, immunomodulatory and anti- allergic activity, as depicted in further in-house study data may synergistically supports cellular resilience and delay aging process. The composition of classical Rasayan along with Mukta, (pearl), saffron and Mushli helps in maintaining overall health, preserving cellular integrity and functional longevity. Collectively, the research demonstrate that the *DRDC/2015/003* possess antiaging effect. The blend of *DRDC/2015/003* consist of multifactorial and synergistic effect which needs to be established through systematic studies.

5. Conclusion

The reduction in H_2O_2 stimulated SA β – gal activity in human fibroblast cells also exhibit potential of preserve cell integrity by decreasing the accumulation of ROS (Reactive Oxygen species) in intracellular space and improves cell viability. Hence, *DRDC/2015/003* exhibits significant protective potential against stress- induced premature cellular senescence or cellular aging.

Abbreviations

DRDC	Dabur Research and Development Centre
HFF	Human Diploid Fibroblasts Cultured
ROS	Reactive Oxygen Species
H_2O_2	Hydrogen Peroxide
ATCC	American Type Cell Culture
USA	United States of America
SA β – Gal	Senescence-associated-beta Galactosidase
DMEM	Dulbecco's Modified Eagle's Medium
g	Grams
µg	Micro Gram
mL	Milli Litre
mM	Millimole

Author Contributions

Baidyanath Mishra: Conceptualization, Funding acquisition, Supervision

Srinivasa Reddy Yathapu: Formal Analysis, Methodology, Project administration, Formal Analysis, Validation

Apratim Sai Rajesh: Methodology, Project administration, Writing – original draft, Writing – review & editing

Divyanshi Priya: Writing – original draft

Conflicts of Interest

The authors declare no conflicts of interest.

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