

IN-VITRO EXPERIMENTAL STUDIES ON SOME ANTI-AGING EGYPTIAN MEDICINAL PLANTS AND IMPACT ON HUMAN HEALTH

By

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Abstract

Aging is a process of physiological deterioration experienced by living organisms with time. The science aspect and discourse are expertly applied for product improvement in response to a rapidly growing demand from the elderly population. The study evaluated some natural medicinal products such as Curcumin, *Aleo-vera*, Coconut oil, Lavender oil, Royal jelly, and *Apis mellifica* honey to detect their efficacy against aging confirmed by targeting enzyme as telomerase using tissue culture cells (BJ). The results showed that these products caused maintenance of the telomere through low activation of telomerase enzyme within the normal range compared with control.

Keyword: TERT, BJ, Honey bee, Royal gel, Curcumin, Lavender oil, Coconut oil, *Alleo-vera*.

Introduction

The WHO definition of health, formulated in 1948, describes health as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (WHO, 2015). Aging is the process that converts young adults, most of them healthy and without assistance from physicians, into older adults whose deteriorating physiologic fitness leads to progressively increasing risks of illness and death (Huber *et al*, 2011). Its effects were so familiar to health professionals and aging adults that they are viewed by both parties as immutable, taken for granted, an arena in which diseases and their treatments take place, but not subject to intervention or modulation (Lombard *et al*, 2016). Scientists are more interested in natural origin pharmacotherapy (Rodrigues *et al*, 2016).

Nowadays, every human being wants to postpone his/her ageing and different theories were given time to time for aging, as to many products available in market, synthetic as well as natural molecule. Thus, it was recommended to highly involved in anti-aging properties of Pharmacophore/ phytochemicals of natural origin (Mondal *et al*, 2015). The science aspect and discourse are expert-

ly applied for product improvement in response to a rapidly growing demand from the elderly population (Stefánsson, 2005). Curcumin cause signaling system which integrates the intracellular regulation of immune responses in both ageing and age-related diseases (Sikora *et al*, 2010). Curcumin's anti-aging properties stem from its ability to delay cellular senescence. Some EOs appear to reveal medicinal properties and are reported to cure one or more diseases and are used in Para-medicinal practices Perry *et al*. (2003) in China reported that LO could show neuroprotective effect in AD model in vivo (scopolamine-treated mice) and in vitro (H₂O₂ induced PC12 cells) via modulating oxidative stress and AChE activity. Hancianu *et al*. (2013) in Romania suggested that suggest that antioxidant and antiapoptotic activities of the lavender essential oils are the major mechanisms for their potent neuroprotective effects against scopolamine-induced oxidative stress in the rat brain. Coconut oil (VCO) has high antioxidant activity can be explained by its high total phenolic content (Marina *et al*, 2009). Royal gel (RJ) raised the average life expectancy, protecting the DNA and lowering the oxidative stress, possible contributor to the longevity

effect of RJ is its enhancement of anti-oxidation capacity and resistance to oxidative stress, which foster scavenging of free radicals (Honda *et al*, 2015). Honey has strong antioxidant activity to prevent several acute and chronic disorders (Ahmed and Othman, 2013). The *Alleo vera* “Elsabaar” stimulates fibroblast activity and collagen proliferation (Thompson, 1991).

Telomeres are defined as dynamic nucleoprotein complexes that are located at the ends of the linear chromosome. Telomerase comprised of two major components, the telomerase reverse transcriptase (TERT) protein and a noncoding telomerase RNA component (TERC). Telomerase is responsible for adding G-rich nucleotide (TTAGGG) sequence to preserve the lengths of telomeres; compensating for the continuous telomere attrition at each cell division (Blackburn and Collins, 2011). Telomerase activity is not adequate to overcome the continuous renewal in adult stem cell, and shortened with aging (Batista, 2014). Telomerase regulation and activity occurred within the cells with various ways, the important of which was transcriptional control of TERT (Bayne *et al*, 2008). TERT is a primary factor that regulated telomerase activity by direct and/or indirect gene expression regulation, alternative splicing, protein tertiary folding, and posttranslational modification (Liu *et al*, 2010). Telomere shortening during aging in human somatic cells resulted from lack of telomerase activity (Blasco *et al*, 1997). To enhance telomere length by increasing telomerase activity was very indicated for anti-aging strategies (Tomás-Loba *et al*, 2008). Functions elucidation of telomerase activity, TERT in neuronal differentiation and survival led to novel approaches to prevent neuronal death and promoting recovery of function in various neurodegenerative conditions (Mattson *et al*, 2001). The TERT was associated with decrease in reactive oxygen species and DNA protection in mitochondria of neurons (González-Giraldo *et al*, 2016).

The present study aimed to evaluate some

Egyptian medicinal plant products (Curcumin, *Aleo-vera*, Coconut oil, Lavender oil, Ro-yal jelly, and *Apis mellifica* honey as anti-aging agents by *in vitro* ELISA assay.

Materials and Methods

The BJ cell line has a long lifespan in comparison with other normal human fibroblast cell lines (ATCC, Biocompare.com), honey bee (*Apis mellifica*), royal gel, and *Aleo-vera*, Curcumin, Lavender oil, Coconut oil (Agriculture Research Center, Egypt). The MTT cell growth assay is a colorimetric assay used for either proliferation or complement-mediated cytotoxicity assays (M-8910, Sigma-Aldrich), and an *in vitro* ELISA for the quantitative measurement of the TERT in human serum, plasma, tissue homogenates (K4187 biovision.com, Bio-vision, Inc.).

Unprocessed Curcumin, Lavender oil, and Coconut oil were brought from a well-known spice dealer. Curcumin (10gm) was dissolved in 10ml Lavender oil and added to 10ml coconut oil and then mixed well to have homogenous to prepare mix-1. As to *Aloe vera* “Alloeh” gel, mature leaves were obtained fresh from The Egyptian Ministry of Agriculture model farms with neither pesticides nor fertilizers. The outer rind was cut off the leaf using a scalpel and the parenchyma was scooped out using a spoon. The mucilage was blended and then centrifuged at 10000g for 3 minutes to remove fibers, and the outcome gel was stored at 4°C. Unprocessed honey was from Ministry of Agriculture model farms without heat processed. Dried Royal jelly (100mg powder) was dissolved in 10 ml honey for use. Mix-2 prepared by adding equal amount of lavender, honey, and royal gel and mixed them until became completely homogenous. Maintenance of cell line were maintained (James and John, 1998) followed by cell counting (Soliman *et al*, 2013). Cytotoxicity Assay using MTT was done on test and observed 24h post treatment (Meerloo *et al*, 2011). The effect of tested materials on fibroblast cell migration (Marshall, 2011) and telomerase enzyme assay using ELISA kit for *in-*

in vitro quantitative determination of Human TERT from Bio-vision.

Statistical analysis: Data were collected, tabulated and analyzed statistically as minimum, maximum, mean value, standard deviation, standard error, and range (Snedecor

and Cochran, 1982). Comparison between groups for significance was done by *t* test.

Results

Details were given in tables (1, 2 & 3) and figures (1, 2, 3, 4, 5 & 6)

Table 1: MTT assay of samples to determine cytotoxicity on fibroblast cells (BJ)

Series	Samples	fibroblast cells BJ				
		concentration /Proliferation %				
		10000ug	1000ug	100ug	10ug	1ug
1	Curcumin	76.75	99.23	96.43	97.64	93.78
2	Coconut oil	72.55	95.58	94.25	93.74	92.36
3	Lavender oil	77.66	99.16	95.18	96.2	90.87
4	Mix-1	87.29	99.64	96.96	99.57	96.67
5	Honey	97.86	99.63	98.04	99.82	92.71
6	<i>Aloe vera</i>	91.66	94.09	92.82	92.82	92.10
7	Royal Jelly	93.82	95.22	94.2	93.21	90.361
8	Mixt-2	96.42	97.77	96.71	98.98	92.50

Table 2: Fibroblast assay of tested substances and mixtures

Series	Samples	BJ at 24h
		Fibroblast cell count /migration
1	Curcumin	73.3±1.62
2	Coconut oil	60±1.59
3	Lavender oil	75.6±2.41
4	Mix-1	86.7±2.59
1	Honey	80±1.84
2	<i>Aloe vera</i>	62.2±1.39
3	Royal jelly	68.8±2.28
4	Mix-2	73.3±2.13

Table -3: Determination of Telomerase enzyme (TERT)

series	compound	TERT ng/ml
1	Curcumin	3.128±0.031
2	Coconut oil	3.048±0.031
3	Lavender oil	2.887±0.040
4	Mix-1	3.294±0.08
5	Honey	2.82±0.038
6	<i>Aloe vera</i>	2.78±0.039
7	Royal gel	2.66±0.014
8	Mix-2	2.73±0.045
9	control	2.586±0.065

Discussion

Aging is the progressive accumulation of changes with time that are associated with or responsible for the ever-increasing susceptibility to disease and death which accompanies advancing age (Harman, 1981). Meanwhile, aging leads to numerous changes that affect all the human physiological systems as the endocrine, nervous, digestive, cardiovascular and muscle-skeletal systems (Müller *et al*, 2019), as well as susceptibility to vector borne infectious diseases particularly with climatic changes. During aging the immune system undergoes remarkable changes that collectively are known as immunose-

nescence, which refers to disruption in the structural architecture of immune organs and dysfunction in immune responses, resulting from both aged innate and adaptive immunity (Thomas *et al*, 2020). In Egypt, the Central Agency for Public Mobilization and Statistics reported that the elderly population reached 6.5 million in 2019 (<https://english.ahram.org/News/351853.aspx>)

Telomerase is a ribonucleoprotein enzyme essential for replication of chromosome termini in most eukaryotes. Expression of catalytic subunit of human telomerase (hTERT), in normal human fibroblasts allowed them to escape replicative senescence. Gorbunova *et*

al. (2003) suggested that excessive telomerase activity may act as a hyper-proliferative signal in cells and that induced a senescent phenotype in a manner similar to that seen following overexpression of oncogenic Ras, Raf, & E2F1. Thus, there must be a critical threshold of telomerase activity that permits cell proliferation. Catalytic component of telomerase holoenzyme complex that main activity is the elongation of telomeres by acting as a reverse transcriptase that adds simple sequence repeats to chromosome ends by copying a template sequence within RNA component of the enzyme (Collins, 2006).

In the present study, showed that the best concentration was the IC₅₀ (10ug) of the tested substances was the safely effective dose, and thus were used throughout the *in-vitro* tests. Majno (1975) stated that the ancient Egyptians utilized honey to treat wounds, which was experimentally demonstrated to be efficacious in promoting healing and preventing infections. Prashar *et al.* (2004) in the United Kingdom tested the cytotoxicity effect of lavender oil on fibroblast cells and endothelial cells, the results showed that 0.125% (v/v) oil concentration caused cells viability of 80-100% at both cell types and increasing the concentration affected the viability of cells. Also, Kim *et al.* (2010) Korea tested the cytotoxicity effect of royal jell on human skin cells by using the MTT cytotoxicity assay. They concluded that RJ enhanced the migration of fibroblasts and alters the levels of various lipids involved in the wound healing process. Ranzato *et al.* (2013) in Italy performed a Calcein-AM assay to evaluate cytotoxicity effect of honey on the fibroblast cells, found that honey of 0.1% (v/v) caused low cytotoxicity levels on the fibroblast cells. Hormozi *et al.* (2017) in Iran reported that tested the effect of *Aleo vera* was dose- and time-dependent on expression of bFGF and TGFβ1 in the fibroblast cell *in-vitro*. Varma *et al.* (2019) reported that the MTT assay results of coconut oil showed that 706.53 & 787.15mg/ml caused 50% to-

xicity to human cells, also IC₅₀ of the coconut oil was determined to be 712.62 mg/ml.

In the present study, determined the fibroblast cells count of tested substances and their mixture and found that the mixture gave the highest value followed by lavender oil, curcumin, and then coconut oil. Zhang *et al.* (2007) in China reported that the curcumin had an inhibitory effect on fibroblast cells proliferation by using *in-vitro* MTT chromatometry. Nevin and Rajamohan (2010) in India examined the coconut oil effect on fibroblast cells by topical application, found that virgin coconut oil on can be attributed to the cumulative effect of various biologically active minor components present in it. On the other hand group-2 representing the results of bee honey, royal gel, *Aloe vera* and the mixture between them (mix-2), it was found that bee honey had the highest value, followed by mix-2, then royal gel, and lastly *Aloe vera*. Du Toit and Page (2009) in South Africa reported that honey-based product showed excellent cytocompatibility with tissue cell cultures compared with the silver dressing, which indicated consistent culture and cell toxicity. Koca Kutlu *et al.* (2013) in Turkey tested the effect of lavender oil on fibroblast cells migration by applying it on injured rat cells, found that transcutaneous electrical nerve stimulation (TENS), saline solution, povidone-iodine, and lavender oil, via expression of growth factors in a rat model of wound healing. Kim *et al.* (2010) in Korea tested the effects of royal gel on the human fibroblast cells through exposing the fibroblast cells to the royal gel, the results showed that the viability of cells was 100% with different concentrations of royal gel, it was also showed that royal gel had a positive effect on the cell migration of fibroblast cells, the performed *in vitro* cell migration assay showed that more than 100% of fibroblast cells was covered and was obviously increased compared to control. They concluded that RJ enhanced the migration of fibroblasts and alters the levels of various lipids involved in wound healing process. Sha-

faie *et al.* (2020) in Iran evaluated the angiogenic and migrated effects of *Aloe vera* gel on fibroblasts and endothelial cells. They reported that *A. vera* has a higher proliferative effect on fibroblasts in comparison with endothelial cells. Also, it induced migration of fibroblasts. Integrin α_1, β_1 & PECAM-1 gene expression increased significantly in *Aleo vera* treated fibroblasts and endothelial cells in comparison with the control groups. However, the expression of these genes was significantly higher in fibroblasts in comparison with endothelial cells. Protein levels of PECAM-1 showed no change in both cell types upon *Aleo vera* treatment.

In the present study, curcumin, coconut oil, lavender oil, and their mixture (mix-1) gave the highest value, followed by curcumin, then coconut oil, and lastly lavender oil attributing to the synergism effects between them. Xiao *et al.* (2014) in China reported that Alzheimer's disease is a consequence of an imbalance between the generation and clearance of amyloid-beta peptide in the brain. They measured cell survival and cell growth, intracellular oxidative stress and hTERT expression. After RNA interference, the effects of curcumin and Cur1 on cells were verified Cur1 gave stronger protective effects than curcumin. However, when telomerase was inhibited by TERT siRNA, the neuroprotection by curcumin and Cur1 were ceased. They concluded that the neuroprotective effects of curcumin and Cur1 depended on telomerase and telomerase may be a target for therapeutic effects of curcumin and Cur1. Bei (2017) tested the effect of coconut oil on telomerase enzyme by diet supplementation, found that the coconut oil neither had effect on the telomerase enzyme nor showed any change on its regulation, coconut oil is mainly composed of saturated fatty acids, other studies showed that there is no link between saturated fatty acids in take and the telomerase enzyme, which explains why coconut oil showed no effect after its supplementation. Plant (2016) evaluated 31 commonly-used therapeutic essential oils by

PCR-based telomere length assay, and verified by qualitative cytogenetic analysis, found that gene expression analysis revealed that these oils didn't cause a concomitant increase in the expression of hTERT, the gene coding for catalytic subunit of the telomerase protein complex, but instead down-regulated the TERF-1 telomere length suppressor. He concluded that essential oils have potential use as a supplement to impact cellular senescence, once this mechanism was fully elucidated. Abd El-Hack *et al.* (2021) in Egypt highlighted curcumin's chemical properties, allowing the people to expand their perspectives on its use in functional products development with the health-promoting properties. Hamed *et al.* (2022) in Egypt reported that curcumin can be of value as an adjuvant therapy to conventional antiparasitic agents and also give promising results when used alone at higher doses.

In the present study, the effect of *Aleo vera*, honey bee, royal gel, and their mixtures on telomerase enzyme showed that honey bee had the highest value followed by the mixture, *Aleo vera*, and lastly royal gel. The results proved the leading effect of honey bee. Nasir *et al.* (2015) in Malaysia tested honey effect on telomerase enzyme by checking the telomeres length in bee keepers who consume honey for long time periods, which showed the longer telomeres length in the bee keepers is associated with the longer period of honey consumption that confirmed the positive effect of honey on telomeres length and telomerase enzyme. El-Kased *et al.* (2017) in Egypt reported that 75% honey-chitosan hydrogel possesses greater wound healing activity compared to commercial ones and safely used as an effective natural topical wound healing treatment Jenkhetkan *et al.* (2017) tested the effect of royal gel on telomerase activity, and showed that treating the cells by royal gel at low doses caused maximum benefits. But, they added that highest dose; can harm human cells by inducing genotoxicity, increasing ratio of cell death while lowering the ratios of cell sur-

vival and cell proliferation. Wang *et al.* (2020) in China showed that the telomeres length was shortened with the increasing *Aloe vera* concentration, and activity of telomerase enzyme was reduced compared to negative control and that the hTERT mRNA was reduced as *Aleo vera* concentration increased. They concluded that *Aleo vera* inhibited the activity of telomerase enzyme by decreasing the total hTERT protein amount; transcription suppression linked to demethylation of hTERT gene promoter. Fernandes *et al.* (2021) in Brazil found that neural cells underwent functional or sensory loss due to neurological disorders. They added that essential oils of six medicinal plants species gave positive responses in neurological disorders; anxiety, dementia, oxidative stress, cerebral ischemia, and oxidative toxicity.

Conclusion

Many natural products are increasingly used worldwide for health benefits by controlling oxidative damage generated by free radicals and serve as a free radical scavenger. The outcome results proved that the *Aloe vera*, Coconut oil, Curcumin, Honey, Lavender oil, and Royal Jelly acted as anti-aging by acting on the fibroblast cell and telomerase activity leading to maintain telomere length through their anti-oxidative activities.

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Explanation of figures

Fig. 1: Percentage of proliferation for samples from each concentration as in table 1.

Fig. 2: Percentage of proliferation for samples at each concentration as in table 1.

Fig. 3: Fibroblast assay of tested materials and mixture of them

Fig. 4: fibroblast assay of tested materials and mixture of them.

Fig. 5: TERT assay of tested materials and mixture of them.

Fig. 6: TERT assay of tested materials and mixture of them

