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Modulation of Telomerase Gene in Liver Cancer by Natural Compounds

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Abstract: Until now, the focalization of liver diseases has remained a mystery. Although many patients have risk factors such as viral hepatitis B&C, alcohol consumption, drugs and fatty liver disease, but little number of patients has no obvious risk factors. Telomere shortening limits, hepatocyte regenerative potential during chronic liver dysfunctions, and cirrhosis is linked with hepatocellular senescence. Shortening of Telomere and its ordinance plays a critical role in tissue regeneration during ageing, chronic disorders, and tumor progression and promotion. Telomere shortening is arrested by a special type of holoenzyme called telomerase whose task is to carry out the end replication by de novo synthesis of telomeric repeats. To target this holoenzyme, recent evidence suggests that certain natural compounds potentially suppress the transcription of hTERT (human telomerase reverse transcriptase) subunit. Therefore, this property can be utilized efficiently for the treatment of cancer. This review summarizes current knowledge about telomerase complexes and tries to give an update on telomerase expression modulation in response to certain natural compounds in liver cancer.

Index Terms: Hepatocellular Carcinoma (HCC), Liver cancer, Natural compounds, Telomere, Telomerase.

I. INTRODUCTION

HCC (Hepatocellular carcinoma) is the most frequently occurring primary liver tumour and the leading cause of death worldwide (Balogh et al., 2013). The presence of HCC frequently complicates the natural antiquity of cirrhosis (Schuppan&Afdhal,2008). Several studies have discovered that telomere shortening is a common feature of chronic liver disease, raising the possibility that it contributes to HCC pathophysiology (Carulli& Anzivino, 2014). Telomere shortening and regulation of telomerase are important in tissue regeneration as we age, chronic disease promotion, and cancer progression (Bernardes & Blasco,2013).Telomere shortening limits hepatocyte regenerative capacity in chronic liver disease, resulting in regeneration of cell, exhaustion, cirrhosis formation &fibrosis (Bernardes &Blasco,2013).

Shortening of telomeres decrease the risk of HCC and slow cancer progression (Carulli & Anzivino, 2014). According to such reports, the therapeutic option of telomerase reactivation to prevent the exhaustion of the liver's regenerative tendency may be beneficial. To better understand the biology of telomeres in human disease,carcinogenesis, and potential therapeutic options, we must first define telomeres and telomerase.

Telomeres are repeating sequences of nucleotide found at the chromosomes' end that help to maintain genomic integrity. Normal cells' progressive shortening causes chromosomal instability, which is regulated by a cellular reverse transcriptase, the telomerase-incorporated enzyme in cancer cells (Carulli & Anzivino, 2014; Nakamura et al., 2001).Telomeres are structures made up of DNA and proteins found at both termini of chromosomes that safeguard genome from nucleolytic degradation, inter-chromosomal fusion, unneeded recombination and repair, (Carulli & Anzivino, 2014). Telomeres are made up of G-rich telomere sequences with associated proteins. In humans, the 5'-TTAGGG-3' telomere strand forms a single-stranded overhang 100-200 bp in length (Palm & Lange, 2008).

The telomeric DNA strand folds back to form the Telomere loop (T-loop), which acts as a cap to protect the single-stranded overhang from degradation (Pfeiffer & Lingner,2013). Telomeres can also form secondary DNA conformations, such as intermolecular G-quadruplexes, which reduce telomerase activity. In addition to these DNA structures, the telomere is associated with several DNA binding proteins. The "Shelterins"

are a group of six proteins (TRF1, TRF2, POT1, TIN2, TPP1, and RAP1) that are required for telomere length regulation and integrity (Palm & Lange,2008).

Telomerase is a ribonucleoprotein that catalysis chromosomal telomere elongation in eukaryotic cell division and is particularly active in cancer cells (Carulli & Anzivino, 2014). Human Telomerase RNA (hTR) and the catalytic protein human Telomerase Reverse Transcriptase are the two main components of human telomerase (hTERT). Other proteins, such as dyskerin (DKC1) and chaperones interact with and regulate the telomerase complex (Cohen et al., 2007). The human telomere sequence is complementary to the hTR template, allowing it to recognize and anneal to the telomeric DNA strand. The complementary hTR templates are then reverse transcribed by hTERT, and the telomeric sequence is synthesized.

Telomerase is activated and expressed in most tissues during embryogenesis, but it is silenced in adult somatic cells. Nonetheless, activity of telomerase is expressed in extremely proliferative tissues as in stem cells, activated lymphocytes, and germ cells to stabilize telomeres. Telomerase is also present in abundance in cancer cells, granting them immortality and the ability to replicate indefinitely. Telomerase-negative somatic cells express hTR and DKC1, but not the catalytic subunit hTERT; telomerase-active immortal cells express hTERT. Human telomerase is thus an important determinant of telomerase activity, and approximately 90% of all tumors have detectable telomerase activity (Shay &Bacchetti,1991).As a result, if cancer cells' telomeres are turned off, they will shorten until they reach a 'critical length.' This would stop cancer cells from uncontrollably dividing and forming tumors (Pfeiffer et al., 2013).

Telomerase activity has been discovered in approximately 90% of all tumor samples (Kim et al.,1994). Recent studies demonstrated that telomerase expression is sufficient for cells to pass through the two proliferation barriers (M1 and M2) and immortalize a wide range of cell types. Telomerase expression causes tumorigenic transformation of normal human epithelial cells and fibroblasts in collaboration with several oncogenes (Shay et al., 1991). Telomerase's role in this process is to provide infinite replicative potential.It may not be necessary if young cells with adequate proliferative capacity are used. Furthermore, in immortal cells it cause telomere shortening, telomerase inhibition and apoptotic cell death (Kim et al.,1994).All of these findings suggest that telomerase activity is almost universally required for cancer cells' cellular immortalization andto proliferate indefinitely. Recognizing the Telomerase regulation mechanism,would undoubtedly have significant implications for human cancer research and management.

Telomere structure and its associated proteins:

Telomere structure is made up of G-rich telomere sequences as well as associated proteins. The 5'-TTAGGG-3' telomere strand in humans forms a single-stranded overhang 100-200 bp in length (Shay et al.,1999). The telomeric DNA strand folds back to form the T-loop (Telomere loop), which acts as a cap to protect the single-stranded overhang from degradation (Hahn et al., 1999 & Griffith et al., 1999). Telomeres can also adopt secondary DNA conformations like intermolecular G-quadruplexes, which can reduce telomerase activity (Stansel et al., 2001). Furthermore, to these DNA structures, the telomere is associated with several DNA binding proteins. The "Shelterins" are a group of six proteins (TRF1, TRF2, POT1, TIN2, TPP1, and RAP1) that are required for telomere length regulation and telomere integrity (Parkinson et al., 2002; Palm &Lange, 2008). Due to the heterochromatic structures surrounding the chromosome end and the non-coding telomere sequence, initially, telomeres were thought to be transcriptionally silent because of a "telomere position effect," genes near the telomere and in sub-telomeric regions are frequently silenced (Raynaud et al., 2008). Recent research has revealed that telomere DNA transcribes to TERRA (Telomere repeat containing RNA), a non-coding RNA that appears to be involved in regulating telomere integrity (Raynaud et al., 2008 & Baur et al.,2001).

The telomere hypothesis of aging and immortalization:

In human somatic cells, telomere length (TL) ranges from 4 to 14 kb and varies depending on a variety of factors such as heredity, cell type, age, and epigenetic influences. The TL also varies between chromosomes (Baur et al.,2001). Telomerase hypothesis of cellular ageing and immortalization demonstrated a close relationship between telomere length, replicative capacity, and cellular senescence in in vitro fibroblast cultures (Baur et al., 2001). With each population doubling, the average normal somatic cell loses approximately 50bp of telomere sequence due to lack of telomerase activity. After a certain number of population doublings, cells enter replicative senescence in culture at a critical length of telomere called as the Hay flick limit or stage of death 1 (M1-stage) (Azzalin et al., 2007).

At M1-stage, cells have a critical telomere length of approximately 5-6 kb long, and show cell cycle arrest, low metabolic rates, and exhibit -galactosidase expression together with morphological changes (Allsopp et al., 1992). The M1-stage can be bypassed by inactivating tumor suppressor genes (p53 and, pRb) (Shay et al., 1991) leading to further cell divisions. As a consequence, cells divide further with progressive telomere loss.

Finally, the cells reach Mortality stage-2 (M2-stage) or “Crisis’’, where a massive cell death occurs, and telomeres are extremely short. At this point, telomeres are no longer protecting the chromosome ends, and signs of chromosomal instabilities such as anaphase bridges and chromosome fusions are frequently observed (Wright et al., 1989). Cells can occasionally overcome crisis and attain immortal status by counter-balancing the unprotected telomeres with de novo formation of telomere

tracts, either through telomerase activation or by alternative telomere lengthening (ALT) (Dimri et al., 1995). In cell types other than the fibroblasts used to elucidate the telomere hypothesis, differences appear in the senescence stages and checkpoints, indicating that the immortalization process is somewhat specific to cell type (Fridman& Tainsky, 2008).

Telomerase and cancer

Transformed cells are immortalized during tumorigenesis, and required necessary steps for unlimited replication. Immortalization is attained by activation of telomerase and hTERT in vast majority of cases. hTERT activity is sufficient to immortalize cells, but is not sufficient for transformation (Blackburn et al.,2006). Normal human epithelial cells and fibroblasts are transformed by co-expression of hTERT, SV40, and H-ras. Telomerase activity is detected in 85-90% of malignancies, and a high level of telomerase has been considered a negative prognostic marker in various tumor types (Greider, C.W, 1996).

In the mid-1990s, researchers proposed that telomerase upregulation as well as re-expression is a major key event accountable for cancer cell growth(Artandi & DePinho, 2010). Normal cells have a gradual mitosis-related erosion of telomeres that eventually limits replicative life span, whereas cancer cells have active telomerase and no loss of chromosomal ends. It was hence proposed that for cells to avoid replicative senescence and continue to proliferate indefinitely, telomere stabilization is required (Vaziri,H, 1993).

The vast majority of tumor biopsies contained telomerase activity 85 percent (N.W. Kim, 1994). Furthermore, telomerase-positive cell lines are usually immortalized by either naturally or after infection with oncogenic viruses such as human papillomavirus types 16 and 18or simian virus 40. (Hahn et al.,2002).These findings support the theory that telomerase is highly activated during immortalization in vitro and tumorigenesis in vivo. In immortal cell lines, however, telomerase activity is not always detectable.(Hiyama & Hiyama,2003).The overwhelming majority of evidence suggests that normal somatic cells lack telomerase, whereas stem cells and germ cells in renewable tissues. Normal cells may contain a telomerase inhibitor, whose inactivation or deletion is needed forimmortality, longevity and Cancer-causing transformation (Dhaene et al.,2000). The activity of telomerase has also been discovered in non-cancerous cells or tissues that are highly proliferative, such as the epidermis' basal layer, oral mucosa and endometrial tissue during the menstrual cycle's proliferative phase (Dhaene et al., 2000). These new findings debunk a popular theory that telomerase activation occurs only during tumorigenic transformation.Rather, they hypothesized that telomerase activity is also more closely linked to cell proliferation(Belair et al.,1997).

Telomerase activity has been shown to be a cell proliferation marker rather than a cancerous transformation in both normal and tumorous human uroepithelial tissues (Dhaene et al.,2000). They demonstrated that normal cells can express telomerase activity under in vitro proliferative conditions. Telomerase was not found in normal human uroepithelial cells, uncultured (HUCs).When the same cells were grown in vitro as proliferating cultures, telomerase activity was detected, though at lower levels than tumor+ cells (Dhaene et al .,2000). Transfection result with hTERT has conclusively demonstrated that hTERT is the rate limiting factor for elongation of telomere (Bryan et al., 1995). As most human somatic cells lack the telomerase subunit reverse transcriptase but have all of the other enzyme components, expressing the missing hTERT component results in enzyme reconstitution.In pre-crisis cultures of human vascular endothelial cells, telomerase-negative retinal pigment epithelial cells, old fibroblasts young/midlife the hTERT gene was transfected, resulting in increased telomerase activity, telomere elongation, and replicative growth that lasts indefinitely,establishing a causal link between telomere shortening and cellular senescence in vitro. While telomerase ectopic expression was sufficient for immortalization, it did not cause malignant transformation-related changes such as loss of contact inhibition, increased growth rate, disruptions in the pRB and p53-mediated cell cycle checkpoints, acquisition of serum independent growth, or cytogenetic abnormalities, indicating that telomerase expression is not oncogenic. (Bodnar et al., 1998).

Furthermore, full-length hTERT expression has been linked to the progression of HCC tumors and has a positive correlation with cMyc (Vaziri & Benchimol, 1998). As hTERT appears to play a role in HCC progression, it could be used as a biomarker for the disease, and the pathways that lead to hTERT activation could be used as therapeutic targets.Activity of telomerase is regulated during normal human growth and development to meet the proliferative demand of specific cellular functions while also preserving proliferative barriers (senescence) against tumorigenesis. Senescence has been proposed as a tumor suppressor mechanism to prevent the accumulation of multiple oncogenic mutations (Jiang et al., 1999).

Telomerase activity has been found in approximately 90% of tumor samples (Kim et al., 1994). Telomerase expression is sufficient for cell escape from (M1 and M2) the two proliferation barriers and for the immortalization ofmany cell types, according to recent experimental data. (Shay et al., 1991 & Kim et al., 1994).

Telomerase expression causes direct tumorigenic transformation of normal human epithelial cells and fibroblast cells in collaboration with several oncogenes (Hahn et al., 2002). The specific function of telomerase during this process is to provide an infinite replicative potential. It may not be necessary if young proliferative cells are used. Furthermore, telomere shortening and apoptotic cell death are caused by telomerase inhibition in immortal cells (Fan et al., 2005). All of these findings point to the fact that telomerase activity is almost universally required for cancer cells to be immortalized and

proliferate indefinitely. Understanding the mechanisms of telomerase regulation would have far-reaching implications for human cancer research and management.

Effect of metallic and natural compounds on telomerase.

A. Telomerase modulators.

(1) Arsenic

Arsenic and its derivatives were previously used to treat diseases such as diabetes, psoriasis, syphilis, skin ulcers, and joint diseases. As is now widely used in the treatment of patients suffering from acute promyelocytic leukemia. International Agency for Research on Cancer (IARC) has identified arsenic as a carcinogenic element based on epidemiological studies, but it is also used in the treatment of neoplastic diseases, as previously stated (Kulik et al., 2016). In patients with mild side effects, arsenic trioxide (As2O3) injection has been used to treat primary liver and gallbladder cancer (Qian et al., 2001).

Arsenic strongly inhibits the transcription of the human telomerase gene's reverse transcriptase subunit (hTERT). Arsenic at its recommended concentration has been shown to be effective in the treatment of various diseases like acute promyelocytic leukemia although its higher concentration is carcinogenic in nature. According to recent research, As2O3 inhibits cancer cell growth and induces apoptosis more in liver cancer cells than in normal cells (Sadaf et al., 2018). It has also been reported that arsenic exposure causes chromosomal abnormalities with a high prevalence of end-to-end fusions and inhibits hTERT gene transcription. These chromosomal end fusions suggested that arsenic may inhibit telomerase activity (Chou et al., 2001). Telomerase activity declines, resulting in chromosomal end lesions that promote genomic instability, carcinogenesis, or cancer cell death (Chen et al., 2007).These phenomena may explain arsenic's seemingly contradictory carcinogenic and antitumor effects.

(2) Selenium

Selenium is frequently referred to as a metalloid. It moves through the aquatic environment similarly to arsenic and antimony; at higher concentrations, its water-soluble salts have a toxicological profile similar to arsenic. In cadmium-transformed 16HBE cells, selenium inhibits telomerase activity (Chen et al.,2007). After 24 hours of selenium exposure, hTERT and c-myc mRNA expression decreased, but mad1 mRNA expression increased (Ramlee et al.,2016). Selenium inhibits telomerase activity by decreasing hTERT and c-myc mRNA expression while increasing mad1 mRNA expression in cadmium-transformed 16HBE cells, and selenium concentration correlates significantly with these changes (Chen et al.,2007).

(3) Cadmium (Cd), Chromium (Cr), Iron (Fe), and Manganese (Mn)

Several studies have linked heavy metals such as Cd, Mn, Cr, and Fe to the formation of reactive oxygen species (ROS). Because telomere GGG triplets are sensitive to hydroxyl radicals, oxidative stress has a greater impact on telomere shortening with end-replication. Increased ROS production can trigger other signals, worsening oxidative stress and leading to a variety of diseases (Ko et al., 2017).

B. Telomerase inhibitors

(1) Butein from medicinal plant butea monosperma.

In the context of telomerase regulation, several natural-derived compounds were discovered to have antitumor activity (Holysz et al.,2013). Butein (3,4,2′,4′-tetrahydroxychalcone), a polyphenolic compound that inhibits telomerase by suppressing TERT gene expression, is one of them. Butea monosperma, a well-known medicinal plant in India and the tropics, yielded this compound. Furthermore, the suppression is c-myc-dependent, because butein prevents c-myc from binding to the TERT promoter, resulting in lower TERT expression. In contrast toalternative lengthening of telomerase (ALT) cells and telomerase-positive, ALT cells had lower c-myc activity but no difference in gene expression (Holysz et al., 2013). As a result, a new mechanism for butein's anti-proliferative effect on liver cancer cells is proposed.

(2) Pectenotoxin-2 from marine sponges and shellfish

Another compound, the most toxic of the pectenotoxins (majorly present in marine sponges and shellfish), pectenotoxin-2 (PTX2), has been shown tosuppress TERT gene expression & telomerase activity at the transcriptional level (Kim et al., 2013). Suppress telomerase activity at the transcriptional level by suppressing TERT gene expression (Kim et al., 2008). The suppression of Sp1 binding and c-myc on TERT regulatory regions was demonstrated to be mediated by PTX2-dependent TERT expression attenuation. Both factors have a post-transcriptional effect on telomerase by decreasing Akt phosphorylation and thus TERT phosphorylation and nuclear translocation.The effects of PTX on MDA-MB-231 and MCF7 (human breast cancer cells) revealed a significant induction of G2/M phase arrest via down regulation of cyclin B1expression and cdc2 as well as cdc25c phosphorylation leads to significant inhibition of cell proliferation(Joseph et al., 2010). Several studies also found that over-expression of c-Myc increased the abundance of survivin and T cell differentiation from hematopoietic stem cells (Joseph et al., 2010).Heaphy et al. found that telomere length was shorter in more aggressive subtypes of breast cancer, such as luminal B, HER-2-positive, and triple-negative cancer, implying that tumor telomere length ma0y be useful as a prognostic and/or risk marker for breast cancer (Heaphy et al., 2011; Holysz et al., 2013). When pancreatic and breast tumor cell lines were treated in vitro with

this potent telomerase inhibitor, telomerase activity and cancer stem cell subpopulations was restricted, according to Joseph et al. After 4 weeks of treatment, in vitro treatment with PTX reduced the self-renewal and proliferative potential of MCF7 mammospheres leads to cell death (Joseph et al., 2010).

(3) Genistein from soybean

Genistein has also been identified as one of the most beneficial natural compounds for telomerase (Li, et al., 2009). This natural isoflavone found in soybean products has been shown to inhibit cancer cells, reduce telomerase activity, and aid in apoptosis (Tuli et al.,2019). It was discovered that genistein inhibits TERT transcription in a time- and dose-dependent manner in various cancer cells. Three major DNA methyltransferases (DNMTs) were downregulated in genistein-treated breast cancer cells, suggesting that this compound may suppress TERT through epigenetic mechanisms (Holysz et al., 2013). As a result, it was deduced that genistein may work, at least in part, through epigenetic mechanisms of telomerase inhibition and may facilitate approaches to breast and liver cancer elimination and treatment in combination therapy (Tuli et al.,2019).

(4) Amygdalin from apple seeds

Amygdalin is a cyanide-containing compound extracted from apple, cherry, peach, pear, and plum seeds. Despite the fact that amygdalin-treated cancer cells had lower expression of TERT and TERC than control cancer cells, telomerase activity was also down-regulated (Ji et al., 2015), there was no difference in telomerase activity between control and amygdalin-treated cancer cells. As a result, amygdalin treatment suggests a valuable alternative for cancer research, causing cell growth inhibition and reduction of telomerase activity in human cancer cell lines by increasing -glucosidase activity (Shi et al.,2019). When compared to normal cells, cancer cell lines (MDA-MB-231, MCF-7,A-549and U87-MG) had higher levels of -glucosidase activity for cyanide delivery (Syrigos et al., 1998).Amygdalin-treated cancer cells had a higher frequency of -galactosidase activity than untreated control cells, but there was no difference between control and amygdalin-treated MRC-5 fibroblasts (Liu & Mason,2010). Thus, a new mechanism of amygdalin's anti-proliferative effect on liver cancer cells can be explored (Liu & Mason,2010).

C. Telomerase activators

(1) Essential oils

When administered in low (sub toxic) doses, therapeutic essential oils such as basil (Ocimum basilicum) and rosemary (Rosmarinus officinalis CT cineol) have been shown to increase the apparent length of telomeres in cell culture (Plant J,2016). This effect was discovered with the help of a previously described PCR-based telomere length assay, which was supported by qualitative cytogenetic analysis. These oils inhibited the TERF-1 telomere length suppressor rather than increasing hTERT expression, the gene encoding the catalytic subunit of the telomerase protein complex. More research is required to fully comprehend the mechanism of action of essential oils, which could be used as a supplement to influence cellular senescence.

(2) Ginkgo biloba leaf extract

Ginkgo biloba extract increases telomerase activity and leads to phosphorylation serine/threonine protein kinase Akt which is a downstream effector of PI3K (phosphoinositide 3-kinase. Furthermore, pre-treatment with LY294002 which is a PI3K inhibitor reduced the telomerase activity induced by Ginkgo biloba extract significantly. The findings suggest that Ginkgo biloba extract delays EPC (endothelial progenitor cell) senescence, which is thought to be linked to telomerase activation via the PI3k/Akt signalling pathway.Ginkgo biloba extract may improve EPC functional activity by inhibiting senescence in vitro, paving the way for potential cell therapy (Dong et al., 2007). The list of telomere inhibitors and activators is summarized in Tables I and II.

Discussion and Conclusion

Hepatocellular carcinoma (HCC) is the most typical type of primary liver cancer, and it is linked to conditions that cause chronic liver injury, such as hepatitis virus infection and alcohol intoxication.Telomerase activity is detected in 85-90% of malignancies, and a high level of telomerase has been considered a negative prognostic marker in various tumor types. hTERT is therefore a key determinant of telomerase activity which is sufficient to immortalize the cancer cells. Current treatment modalities, including surgery and liver transplantation offer limited survival benefits. New therapies are desperately needed. The telomerase gene can be targeted as the novel therapeutic approach for the treatment of cancer. Understanding the regulatory mechanism of telomeraseundoubtedly have significant implications for human cancer research and management.

Telomerase is expressed in several human cancers, and its expression is reduced in most normal cells, suggesting that the enzyme could be a good target for anticancer drugs. Most existing anticancer therapies, on the other hand, disrupt both normal and malignant cells and frequently cause serious side effects (Agbarya et al., 2014 & Wright et al., 2001). To mitigate this negative effect, we examined herbal molecules for telomerase regulation as shown in (Fig-1) in order to limit telomerase-producing tumors as well as in understanding telomerase activity, which could provide new insight into a variety of serious clinical issues that we face as societies age.

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Conflict of Interest

No potential conflict of interest, financial or otherwise.

T**able 1: Telomerase inhibitors**

Serial no	Compounds	Natural sources	Effect on telomerase	Pathways if known	References
1.	Butein	Flower of Butea monosperma	Inhibitor	Prevents binding of c-myc to TERT promoter	Moon et al.,2009 [42]
2.	Pectenotoxin-2	Marine sponges and shellfish	Inhibitor	Suppression of TERT gene	Kim et al.,2008 [32]
3.	Genistein	Soybean	Inhibitor	Inhibit the transcription of TERT	Phipps et al.,2009 [47]
4.	Amygdalin	Apple seeds	Inhibitor	Increased β-glucosidase activity leads to a decrease in telomerase activity.	Ji-Yoon et al.,2015 [29]
5.	Gefitinib	Soybean products	Inhibitors	TERT phosphorylation and translocation to the nucleus were inhibited after Akt activation.	Moon et al.,2009 [42]
6.	Sulforaphane	Cruciferous vegetables	Inhibitor	Reveals histone deacetylase inhibition	Meeran et al.,2010 [41]
7.	All-trans retinoic acid	Dark or yellow vegetables and carrots.	Inhibitor	TERT promoter histone H3-lysine 9acetylation (H3-K9-Ac) decreases rapidly.	Phipps et al.,2009 [47]
8.	Natural metals(Cd, Mn, Cr, and Fe).	welding fume	Inhibitor	End-replication has an effect on telomere shortening .	KO, Jiunn-Liang et al.,2017 [34]
9.	Arsenic	Ground water	Inhibitors	Inhibition of human telomerase reverse transcriptase subunit.	Chou et al.,2001 [14]
10.	Selenium	Soils contents	Inhibitors	By lowering hTERT and c-myc mRNA levels.	Chen Het al.,2007 [13]

Table I-Telomerase inhibitors from natural sources with their mechanism of actions.

Table 2: Telomerase activators

Serial no	Compounds	Natural sources	Effect on telomerase	Pathways if known	References
1.	Essential oils	Rosemary (Rosmarinus officinalis) and basil (Ocimum basilicum) oils	Activator	Downregulate the TERF-1 telomere length suppressor.	Plant et al.,2016 [48]
2.	Ginkgo biloba leaf extract	Ginkgo biloba tree	Activator	Telomerase activity and phosphorylation of the serine/threonine protein kinase Akt were both significantly increased.	Dong et al.,2007 [18]
3.	Leptin	● Oatmeal., Grapefruit, Fish, Green tea, ● Broccoli.	Activator	The phosphorylation of activator transcription 3 (STAT3) significantly inhibited leptin-induced hTERT transcription &protein expression	Ren et al.,2010[52]
4.	TA65 / Cycloastragenol (from Astragalus**)**	Traditional Chinese medicines.	Activator	Significantly induces Telomere lengthening.	Salvador et al.,2016 [54]

Table II- Telomerase activators from natural sources with their mechanism of action.

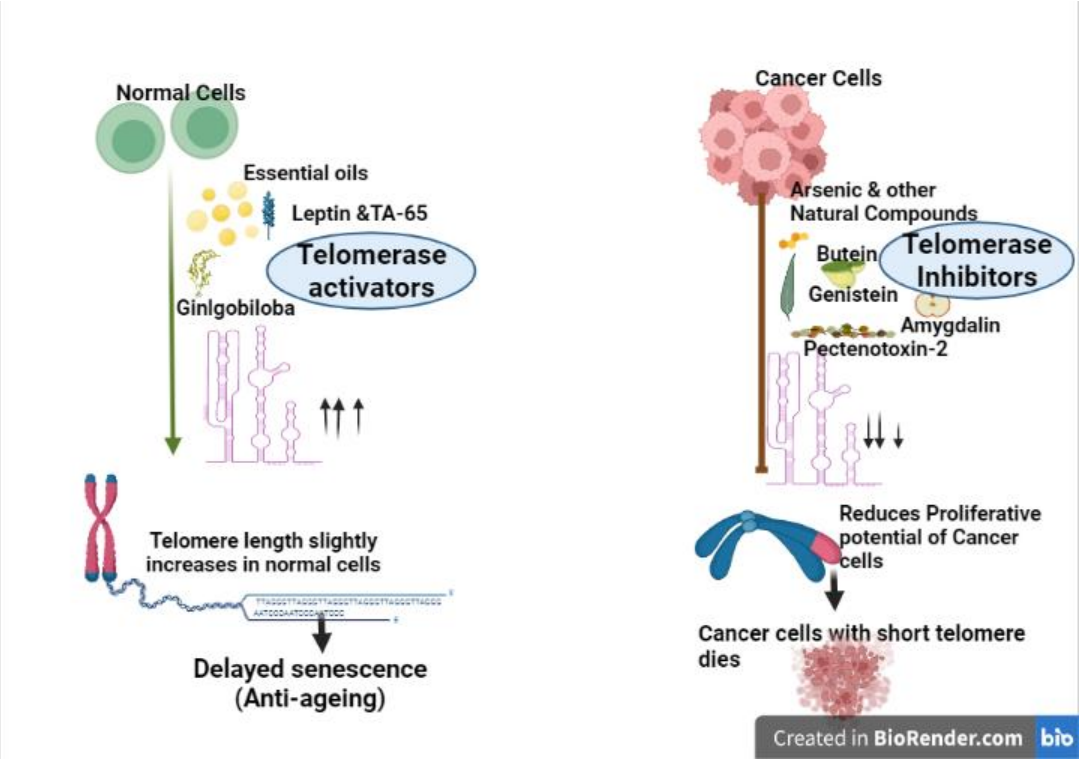

Figure.1-Mechanism of Telomerase regulation in response of natural products. Telomerase activators leads to increase telomere length of normal cells that ultimately results in delayed senescence –anti ageing approach, while on the other hand telomerase inhibitors leads to reduction in telomere length and hence cancer cells with short telomere dies-anticancer approach.

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