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Antioxidant and Anticancer Activity of the Pomegranate and Their Role in Cancer Prevention and Therapy

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Abstract: **Cancer is the second leading cause of death and in now a days Vegetable and fruit consumption is inversely associated with cancer incidence and mortality and Antioxidants from fruits and vegetables have been extensively studied for their free radical scavenging activities to prevent the occurrence of chronic degenerative diseases. The fruits which are high in polyphenols are reported to have antioxidant and chemotherapeutic potential. There is a major need for less toxic but yet effective therapies to treat cancer. Pomegranate has been shown to exert anticancer and antioxidant activity, which is generally attributed to its high content of polyphenols. This review provides that pomegranate targets a broad spectrum of genes and proteins to suppress cancer growth and progression it was shown that pomegranate juice (PJ) and/or pomegranate extracts (PE) significantly inhibit the growth of prostate cancer cells in culture and also inhibit cell proliferation and induce apoptosis in human breast cancer cells (MCF-7), human pancreatic cancer cells, Colon cancer (CACO) and in Hepato-cellular carcinoma (HepGII) cell lines.**

Keywords: **Pomegranate Juice, Breast Cancer, Human Pancreatic cancer, Colon Cancer,Hepato-Cellular carcinoma.**

I. INTRODUCTION

Cancer is the second leading cause of death and is becoming the leading one in old age. It has been estimated that by 2030 the number of new cancer cases will increase by 70% worldwide due to demographic changes alone. The process of cancer development is due to genetic and epigenetic alterations which leads to disruption in basic biological functions, such as cell division, differentiation, angiogenesis.[1],[2],[3],[4],[5]. Pancreatic cancer, one of the most lethal forms of cancer, is the fourth leading cause of cancer death in both genders[6],[7],[8],[9],[10] whereas Prostate cancer (PCa) is the second-leading cause of cancer-related deaths in men in the United States and Breast cancer is the most common form of cancer (other than skin) and the leading cause of cancer mortality among women, next to lung cancer, in the United States.1 Each year, 182,000 women are diagnosed with breast cancer, and 43,300 die; also, 1,600 men are diagnosed with breast cancer, with 400 fatalities[11],[12]. Breast cancer, like many other cancers, tends to spread throughout the body without any symptom. The poor prognosis of pancreatic cancer patients is due to often late stage diagnosis, and the ineffectiveness of current chemotherapeutic regimens[13],[14],[15]. 5-fluorouracil and gemcitabine are the only chemotherapeutic agents that have been successful in the treatment of pancreatic cancer, but their efficacy is low and they cause serious side-effects and in the case of Prostate cancer upto date there is no real cure for the disease beyond surgery and/or radiation when used at early stages of the disease. When recurrence occurs, the cancer can be controlled with hormone ablation therapy (Leuprolide/Lupron®). In addition to the hormone ablation, chemotherapy is available today to treat castration-resistant prostate cancer, but it is not very effective because Prostate Cancer cells divide slowly and, like with prostatectomy, the treatments are aggressive and have many side effects[16],[17],[18]. As a result, researchers are looking for novel strategies to treat Prostate cancer. Inhibitors such as abiraterone (Zytiga ®), have shown great promise as androgen deprivation therapies to prolong overall survival rate among patients with metastatic Prostate Cancer.

Another novel drug, Cabozantinib, is a potent dual inhibitor of the tyrosine kinases c-MET and vascular endothelial growth factor receptor 2 (VEGFR 2), and has been shown to reduce or stabilize metastatic bone lesions in CRPC patients. However, all of these treatments have adverse side effects[19],[20],[21]. It was proved that Vegetable and fruit consumption is inversely associated with cancer incidence and mortality. Currently, interest in a number of fruits high in polyphenolic compounds has been raised due to their reported chemopreventive and/or chemotherapeutic potential. Pomegranate (*Punica granatum*) is a fruit used in many cultures (the genus name, *Punica*, is derived from the Roman name for Carthage, where the best pomegranates were known to grow). The pomegranate tree is native to the region of Persia and is now cultivated over the entire Mediterranean area, Asia, and America. Recent studies have shown that pomegranate is a potent anticancer agent that causes the induction of apoptosis and cell cycle arrest in cancer cells, inhibition of multiple signaling pathways in cancer cells, inhibition of tumorigenesis in animal models of various carcinomas (5-8). The pharmacologicalproperties of pomegranate extracts have been analyzed and the constituents of *P. granatum* include gallocatechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, and phytochemicals, including the punicalagins, a class of tannins unique to pomegranates, which have been shown to possess free radical-scavenging properties, ellagitannins, and other flavonoids like quercetin, kaempferol, and luteolin glycosides[21],[22],[23],[24],[25]. The oil polyphenols in P.Granatum inhibits eicosanoid enzymes, including cyclooxygenase and lipoxygenase. Flavonoids and tannins present in this fruit inhibits cancer cell growth both *in vitro* and *in vivo*.Furthermore, the pomegranate seed oil contains a very high amount of steroids, including esterone, campestrol, estriol, testosterone, stigmasterol. Many of these compounds have cancer chemopreventive properties, in addition to antimicrobial, antiparasitic, antiviral and anticancer properties[26],[27],[28],[29],[30],[31],[32],[33] and *Punica granatum* peel is used to treat infections found in human sexual organs as well as mastitis, acne, folliculitis, pile, allergic dermatitis, tympanitis, scalds.The aim of the present review is to critically discuss the cumulative evidence suggesting that pomegranate consumption possesses multiple biological actions and may be helpful in the prevention and therapy of cancer.

2. POMEGRANATE POLYPHENOLS

Among seed, peel, and juice in the Pomogranate, the peel is the constituent which possesses higher content of polyphenols.This part of the fruit contains large part of ellagitannins[33-50]. Punicalagin, a large polyphenol with a molecular weight greater than 1000, is unique to pomegranate and is part of a family of ellagitannins that includes the minor tannins punicalin and gallagic acid. Punicalagin represents the bioactive constituent responsible for >50% of the antioxidant activity of pomegranate juice. Pomegranate also contains other polyphenols, such as anthocyanins (3-glucosides and 3, 5- glucosides of delphinidin, cyanidin, and pelargonidin) and flavonols. During the juice processing, the whole fruit is pressed and ellagitannins are released into pomegranate juice in significant levels (over 2 g/L juice). In humans and different animal models, it has been found that ellagic acid is metabolized by the colon microflora to form urolithins A and B. Ellagic acid and urolithins can circulate in the blood and reach and accumulate in many target organs, including intestine and prostate, where the effects of pomegranate ellagitannins are observed. It was found that punicalagin-derived ellagic acid is transformed into dimethylellagic acid glucuronide in plasma and urine on the day of administration of pomegranate juice. The anticancer activity of pomegranate is due to the presence of those phytochemical. Two primary mechanisms that have been reported by Punica granatum are cell-cycle arrest and induction of apoptosis.

3. BREAST CANCER

To assess the degree of treatment-induced apoptosis and/or necrosis in MCF-7 cells, the cells were exposed to pomegranate extracts and cultured. Apoptosis was determined by acridine orange/ ethidium bromide nuclear stain, and confirmed by the terminal deoxyribonucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL) assay. TUNEL detects apoptosis-specific DNA fragmentation. Apoptosis was further determined/confirmed by TUNEL, using the ApopTag® kit (Boehringer Mannheim Co., Indianapolis, IN) as previously described. The kit reagents detect apoptotic cells *in situ* by specific end labeling and detection of DNA fragments produced by the apoptotic process. The effects of pomegranate extracts on inhibition of growth and proliferation of human MCF-7 breast cancer cells were determined by using LDH and MTS bioassays[50-55]. Pomegranate extracts inhibited cell growth and decreased cell survival through induction of cell death in both a time- and dose-dependent manner. In the LDH assay, as the concentration of the drugs increased, cells became progressively more cytotoxic, leading to a greater absorbance reading

in the LDH assay and a decrease in absorbance in the MTS assay with a concurrent decrease in percentage of viable cells. Extensive cell death was observed in proliferating human breast cancer cells after treatments with pomegranate extracts. The TUNEL assay showed the typical hallmark morphologic changes associated with apoptosis—which include chromatin condensation, protein breakdown, and DNA fragmentation in the treated cells. Apoptosis, identified as one of the most fundamental biological processes in eukaryotes in which individual cells die by activating intrinsic "suicide" mechanisms, has been suggested to play a key role in damage to cells, caused by a variety of insults. Previous studies demonstrated that pomegranate extracts caused DNA strand breakage in tumor cells and apoptotic cell death in several tumor cells. It has been found to arrest cell cycle progression. Many cancer chemotherapeutic drugs with DNA damage capability are known to induce accumulation of p53 in the cells, indicating cell cycle arrest or programmed cell death (apoptosis).The fact that pomegranate extracts induce apoptosis in MCF-7 cells, as demonstrated here, justifies the inclusion of both phytochemicals in diagnostic and therapeutic strategies in dealing with human pathology[55-60]. Given the results obtained in this study and those from previous experiments, one can speculate that there are substantial possibilities for using both pomegranate extracts as potential tools in human pathophysiology research, especially breast cancer.

4. HUMAN LARYNX EPIDERMAL CARCINOMA

The potent antioxidant and antiatherosclerotic and anti-cancer activities of pomegranate are attributed due to its polyphenols. Ellagitannins (ETs) have also been identified as active antiatherogenic compounds in PJ. It has been shown that pomegranate fruit extracts and its purified ETs inhibit the proliferation of human cancer cells and modulate inflammatory subcellular signalling pathways and apoptosis. The crude aqueous extract derived from the peel of *Punicagranatum* was evaluated as a cytotoxic agent, using the crystal violate assay, and p53 detection and quantitative determination against Hep-2cell line. The results indicated that the extract is potentially cytotoxic to the Hep2 cell line with an inhibition value 73.9%. The cytotoxic assay was measured using the crystal violate stain. This p53 ELISA Kit is a comxlete kit for the quantitative determination of wild- type and mutant p53 in human, mouse and rat samples. In present study the grind extract from pomegranate decreased the viable cell number of Hep-2 cell line[60-65]. In study to investigate the activity of punicalagin, ellagic acid and pomegranate tannin as anti tumor activity they found that these compound from pomegranate have the ability to decrease the viable cell number of human oral, prostate and colon tumor cells; however superior activity was obtained with pure pomegranate juice.

5. HUMAN PANCREATIC CANCER

Pancreatic cancer, is one of the most lethal forms of cancer. Patients with pancreatic cancer face a grim prognosis, as it is highly aggressive and refractory to chemotherapeutics. New treatments are therefore needed. Phytochemicals, the naturally occurring chemicals in fruits and vegetables, have been shown to be effective anticancer agents in laboratory studies and are well tolerated. Pomegranate, being rich in phytochemicals, has been of great interest for the treatment of cancer, and many studies have demonstrated its anticancer effects. A low concentration (≤40 μg/ml) of PE caused the percentage of PANC-1 cells in the G0/G1 phase of the cell cycle to profoundly and significantly increase in a concentration-dependent manner, with an accompanying significant decrease in the percentage of cells in G2. This indicates that PE caused a cell cycle arrest in PANC-1 cells. We demonstrate that PE effectively inhibits the growth and viability of human pancreatic cancer cells by inducing cell cycle arrest, and reduces the tumor-initiating phenotype of the cancer cells. Chemotherapeutic drugs are largely ineffective against pancreatic cancer[65-70]. The reasons for this are not understood, but may result from the presence of multidrug-resistant cancer stem cells which are readily able to repopulate tumors once more differentiated cancer cells are eradicated by the drug. Compared to a clinical achievable concentration of paclitaxel, PE caused a more profound decrease in cell proliferation: >90% inhibition compared to a maximal inhibition of 60% for paclitaxel, and this inhibition occurred more quickly than that with paclitaxel.The phytochemicals responsible for the inhibition of the proliferation of PANC-1 cells are ursolic acid, luteolin and ellagitanins.

6. PROSTATE CANCER

Shortly after these studies were performed, it was found that PE possesses anti-proliferative and pro-apoptotic effects through modulation of cyclin-dependent kinase (cdk) and the cdk inhibitor machinery in PC3 cells. The authors demonstrated that PE inhibited PC3 cell growth by disrupting the cell cycle regulatory molecules in the G1-phase of the

cell cycle. It is well-established that cell cycle progression is regulated by the cyclin and cdk complexes. Cyclins D and E are known to regulate cell cycle progression from G1 to S phase. During the progression of the cell cycle, the cdk–cyclin complexes are inhibited via binding to cdk inhibitors such as the p21 and p27 proteins. These investigators showed that PE significantly down-regulated cyclin D1, D2 and E and cdk2, cdk4 and cdk6 and up-regulated p21 and p27, which may cause a blockage of G1–S phase transition, resulting in a G1-phase arrest and apoptosis [70-80]. Furthermore, apoptosis associated proteins such as cleaved poly(ADP-ribose) polymerase (PARP) and Bcl-2-associated X protein (Bax) were also found to be up regulated in PC3 cells by PE whereas apoptosis blocking proteins such as B-cell lymphoma 2 (Bcl-2) were down-regulated. Bcl-2 is an upstream effector molecule in the apoptotic pathway and is identified as a potent suppressor of apoptosis. Bcl-2 has been shown to form a heterodimer complex with the pro-apoptotic member Bax, rendering it inactive. Therefore, the ratio of Bax to Bcl-2 is a decisive factor and plays an important role in determining whether cells will undergo death or survival. In PE treated cells, the ratio of Bax to Bcl-2 was altered in favor of apoptosis. We showed that 1% or 5% PJ inhibited the growth of PC3, DU145 and LNCaP cells in a dose-dependent manner. Collectively, these results suggest that PE inhibits the growth of PCa cells through cell cycle arrest and stimulation of apoptosis. Because androgen and the androgen receptor play central roles for PCa cell growth and progression, the antiandrogenic effects of PE were studied. It was shown that PE reduces the expression level of androgen biosynthesis genes such as the 3β-hydroxysteroid dehydrogenase type II (HSD3B2) and steroid 5α reductase type I (SRD5A1) genes in LNCaP cells. More recently, PE was shown to reduce the production of testosterone and dihydrotestosterone (DHT) in LNCaP and 22RV1 cells. Therefore, PE may have chemopreventive as well as chemotherapeutic effects against PCa in humans.

7. COLON CANCER

Ellagitannins, derived from PG juice, and their metabolites, urolithins exhibit dose and time-dependent decreases in cell proliferation and clonogenic efficiency of HT-29 cells through cell cycle arrest in the G0/G1 and G2/M stages of the cell cycle followed by induction of apoptosis. Ellagitannins (ETs) and its hydrolysed product, ellagic acid (EA), have been reported to induce apoptosis in human colon cancer Caco-2 cells through down-regulation of cyclins A and B1, upregulation of cyclin E, cell-cycle arrest in the S phase, induction of apoptosis via intrinsic Inflammation plays a key role in the development of colon cancer. In one study, the colonic fibroblasts were exposed to urolithins and ellagic acid, at concentrations achievable after the consumption of pomegranate, with or without inflammatory cytokines, and the effects on fibroblast migration and monocyte adhesion were determined. There was significant down-regulation of inflammatory markers such as PGE2, PAI-1, and IL-8, as well as other key regulators of cell migration and adhesion. Fibroblast migration and monocyte adhesion was inhibited suggesting that consumption of ellagitanin-containing foods has potential beneficial effects on gut inflammatory diseases. Treatment of HT-29 colon cancer cells has been indicated by PG juice through decreasing COX-2 expression and inhibiting inflammatory cell signalling processes which may cause cancer initiation and progression. The beneficial anti-inflammatory effects of pomegranate seed were attributed to punicic acid-mediated down regulation of neutrophil activation and lipid peroxidation. Pomegranate peel extract (6 mg/d) administered to mice over a period of 4 weeks counteracted the high fat-induced expression of inflammatory markers, both in the colon and the visceral adipose tissue. Pomegranate juice significantly down-regulated pro-inflammatory enzymes nitric oxide synthase and cyclooxygenase-2 messenger RNA (mRNA) and protein expression. In addition, it suppressed nuclear factor-κB and VCAM-1 mRNA and protein expression in AOM-treated rats. Pomegranate also inhibited phosphorylation of PI3K/AKT and mTOR expression and increased the expression of miR-126. The specific target and functions of miR-126 were investigated in HT-29 colon cancer cell lines. In vitro, the involvement of miR-126 was confirmed using the antagomiR for miR-126, where the pomegranate reversed the effects of the antagomir on the expression of miR-126, VCAM-1 and PI3K p85β. In summary, therapeutic potentials of pomegranate in colon tumorigenesis were due in part to targeting miR-126- regulated pathways, which contributes in the underlying anti-inflammatory mechanisms. The standardized ellagitanin extracts obtained from pomegranate and berries have been shown to inhibit Wnt signalling, emphasizing further the inhibitory potential of ellagitanin-rich foods against colon carcinogenesis.

8. CONCLUSION

Pomegranate fruit is a powerful exogenous source of antioxidant that has been studied for several pharmacological effects. It has been extensively studied for its antitumor property. Conventional chemotherapeutic agents are expensive and have serious side effects. This has necessitated the need for exploring natural products as alternative

chemotherapeutics or adjuvants to conventional anticancer drugs. Studies have demonstrated a great potential for pomegranate fruit extract as an anticancer agent. In this review, the chemotherapeutic potential of the fruit extract against breast cancer, prostate cancer, pancreatic cancer and colon cancer were discussed. Thus Pomogranate juice can act as the best anti-cancer agent.

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