Chapter VI

Summary and Conclusions
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Cancer is a major public health crisis worldwide including United States and India. Despite advances in the basic as well as clinical investigation related to cancer, it is still one of the dreaded diseases worldwide, and the leading cause of death in developed and second leading cause in the developing world (WHO, 2008). It is important to mention that ~12.7 million new cases and ~7.6 million deaths occurred in 2008 worldwide due to cancer (GLOBOCAN, 2008 Cancer Fact Sheet). At the moment, one in four deaths in the United States is due to cancer. Disparities in cancer incidence and death rates between different geographical regions of the world have been observed and mainly been linked to the life style and socio-economic conditions prevailing in such regions (Figures 1-2 and 5; Jemal et al. 2011 and GLOBOCAN, 2008 Cancer Fact Sheet and International agency for research on cancer). It is observed that developing countries have lower cancer incidences than developed ones (Figure 2 and Table 3), and this disparity has been largely linked to the differential lifestyles including food habits. For instance, Asian countries including China and India have lower risk of acquiring various cancers including prostate and breast, and it has largely been attributed to the abundant consumption of fruits and vegetables.

Incidence rates of cancers in India are lesser compared to the western countries and these rates increase with the increased migration to cities, alterations in life style and increase in life expectancy (Gajalakshmi, et al. 2001; Finlay, et al. 2001). Incidences of head and neck cancers, especially of oral and oesophageal types in India are very prominent but incidence of cancers of prostate, colorectal and lung are lower (Sinha, et al. 2003). Lower cancer incidence rates in India compared to the western world are linked to the frequent consumption of the large amounts of vegetables and fruits in daily diet, and also to the importance of herbs and fruits in the South Asian culture.

Conventional therapies presently employed for cancer control and management are associated with serious limitations of toxicity, dose selection, cost and poor prognosis after treatment. Therefore, search for alternative approaches that are quite safe, least/or nontoxic, cost effective and easily available is desired in this endeavour. The belief that frequent consumption of fruits and vegetables is associated with lower risks of acquiring
some cancers like PCa, lung, breast etc. has led to the identification, isolation and characterization of plethora of novel anticancer agents called “phytochemicals” from fruits and vegetables, and these agents have been shown to offer potent anticancer efficacies against many cancers. Hence, cancer chemoprevention using naturally occurring nontoxic phytochemicals has been suggested as a very good approach in the prevention and management of cancer. Beauty of these phytochemicals is their nontoxicity at recommended doses, easy availability and inexpensiveness than conventional anticancer drugs. Since, cancer is a multistep chronic disease; therefore, phytochemicals have been suggested to be very promising in halting/impeding different stages of this process from initiation to its metastatic progression.

Cancer is a chronic diseases passing through many stages starting from cell transformation (initiation) to tumor angiogenesis and metastasis, and in this process onset of angiogenesis is an important determinant of cancer growth and progression. Solid tumors grow up to ~1-3 mm diameter in size and remain dormant for years owing to the absence of angiogenesis. Initiation of angiogenesis breaks this dormancy and resumes active tumor growth and metastatic progression. Hence, tumor angiogenesis has been advocated as one of the most promising and potential targets in cancer prevention and control strategies. In this regard, large numbers of phytochemicals have been shown to specifically target different aspects of tumor angiogenesis process in their overall anticancer mechanisms (Bhat, et al. 2008).

Prostate cancer (PCa) is one of the leading causes of incidence and second leading cause of death due to cancer in men in United States. Despite advances in understanding the disease, PCa is still refusing to comply with the present conventional treatment modalities due to poor diagnosis, emergence of androgen-independent phenotype at later stages of the disease and poor prognosis after its therapeutic intervention. Therefore, alternative therapies are desired to treat and manage PCa. Epidemiological studies have shown that Asian people have lesser risks of acquiring PCa than western counterparts, and this has mainly been attributed to the frequent consumption of fruits and vegetables by the former. In this regard, in last two decades several phytochemicals have been shown to be effective in halting PCa growth and progression in vitro as well as in vivo at nontoxic doses with mechanism of action defined (Singh, et al. 2006; Mukhtar, et al. 2006).
Therefore, chemopreventive intervention employing natural dietary and non-dietary agents has been suggested as a fruitful strategy in the prevention and management of PCa. Also, many of these agents have been found to be promiscuous in action by modulating more than one target same time (Singh, et al. 2006; Mukhtar, et al. 2007).

Based on the above observations and facts, in current study we tried to test the antiangiogenic and anticancer efficacies of agents, decursin and acacetin using various models. Present studies were taken up based one the already completed studies that have shown that decursin (a coumarin) and acacetin (an isoflavone) possess strong anticancer activities against different cancers like prostate, bladder, colon and breast cancers, T-cell leukemia and lung cancer; but their antiangiogenic effects and associated anticancer mechanisms are yet to be investigated. Herein, we evaluated their antiangiogenic activities in vitro, ex vivo and in vivo that may effectively contribute to their overall anticancer efficacies; and also assessed their anticancer effects on PCa using various standard cell lines including 22Rv1, DU145 and PC3.

We saw that both these compounds possess strong antiangiogenic and anticancer activities using various angiogenic and PCa models. In this study, we show that decursin and acacetin offer strong antiangiogenic activities under the biologically applicable growth conditions employing well accepted models for the first time. We observed that decursin strongly inhibits HUVEC proliferation concomitant with a G1 phase cell cycle arrest, and potently suppresses HUVEC-capillary tube formation and -invasion/migration in a dose-dependant manner that were associated with the repression of matrix metalloproteinase (MMP) -2 and -9 activities by decursin. Decursin also suppressed angiogenesis in ex vivo rat aortic ring angiogenesis model where it powerfully inhibited blood capillary-network sprouting from rat aortic sections. Moreover, decursin potently repressed the expression of various proangiogenic factors like VEGF-R2, VEGF and eNOS in tumor cells which could explain its mode of action as an antiangiogenic agent.

Similarly, we observed that acacetin strongly inhibited growth and survival of HUVECs in regular as well as VEGF-stimulated conditions. It also suppressed capillary-like tube formation by HUVEC on matrigel in a dose- and/or time-dependant manner both in serum as well as VEGF-induced conditions, and furthermore, induced the retraction and disintegration of preformed capillary networks on matrigel. Acacetin
strongly inhibited HUVEC migration and invasion in a dose-dependent fashion. Molecular mechanisms behind this acacetin-induced suppression of angiogenic parameters in HUVEC seem to be accompanied by the inhibition of STAT-1/-3 tyrosine phosphorylations concomitant with the downregulation of VEGF expression, as well as negative modulation of the VEGF autocrine loop by acacetin. Furthermore, acacetin strongly inhibited ex vivo angiogenesis from rat aortic rings (showing complete inhibition at higher dose) and from pre-formed fertilized chicken egg chorioallantoic membrane blood vessels. Moreover, acacetin potently suppressed in vivo angiogenesis in matrigel plug implants employing Swiss albino mice. In addition, acacetin inhibited STAT-1/3 activation (activating phosphorylation) and expression of various proangiogenic factors including VEGF in human prostate carcinoma DU145, PC3, 22Rv1 cells, effects highly desired in suppressing tumor-induced angiogenesis in solid tumor control and management.

There are several published reports showing that decursin and acacetin possess anticancer activities against many cancers including PCa, but their detailed mechanisms of action are yet to be explored. In the current study, we observed that both these agents strongly inhibit cell proliferation, induce cell cycle arrest and inhibit colony forming potential of various PCa cells, and these effects could be associated with the repression/modulation of the expression of various mitogenic, pro-survival and cell cycle regulatory factors in these cells as these pathways mediate such processes. Decursin-mediated induction of G1 arrest in DU145 cells could partly be explained by the increased interaction of CDKI-CDKs, while acacetin-mediated cell cycle arrest induction in these cells could be partly due to the enhanced Rb-E2F interaction induced by this agent. Furthermore, decursin-induced strong G1 arrest and mild G2/M arrest (at higher dose) in 22Rv1 cells could be due to the modulation of various G1-specific factors as well as G2/M-specific proteins in these cells. Both these compounds strongly suppress metastatic attributes like cell adhesion, migration and invasion in various PCa cells that could be due to the modulation of the expression and/or activity of various factors like MMPs, uPA, E-cadherin, N-cadherin and vimentin involved in these processes. Moreover, these compounds possess potential antiangiogenic activities by strongly inhibiting the expression and/or activation of various pro-angiogenic factors in DU145, 22Rv1 and PC3.
cells. Therefore, these results suggest their potential involvement in suppressing tumor angiogenesis and hence, in PCa angioprevention. On the whole, cell cycle progression, mitogenic, pro-survival and metastatic and angiogenic features of PCa are likely targets of decursin and acacetin at various nontoxic doses in PCa, and these targets are considered to be very crucial for cancer chemoprevention and control.

In summary, decursin and acacetin possess promising antiangiogenic and anticancer activities in well accepted models; and hence, could offer promising roles in the cancer prevention and management especially for solid tumors including PCa where the onset of angiogenesis is a critical aspect of tumor growth and metastatic progression. Suppression of STAT activation and concomitant inhibition of VEGF expression could be important mechanism of antiangiogenesis by these compounds as, for example, acacetin suppressed STAT-VEGF axis in both HUVEC as well as tumor cells. Taken together, current findings suggest potential antiangiogenic activities of decursin and acacetin under regular and/or serum-starved but VEGF-stimulated growth conditions, and warrant further pre-clinical studies for their potential clinical usefulness in cancer prevention and management. Furthermore, both these agents show promising anticancer activities against PCa by inhibiting various parameters like cell proliferation, cell cycle progression, mitogenic, pro-survival, and metastatic and angiogenic features that are normally activated in cancer and characteristics of cancerous growth. Therefore, these natural agents could offer potential roles in the prevention and management of cancers including PCa. Furthermore, as decursin and acacetin inhibit expression of VEGF in PCa cells (and also in HUVEC) and this inhibition was concomitant with the suppression of STAT signaling in them (in case of acacetin); consequently, VEGF suppression by these agents could account for their antiangiogenic efficacies in part to their overall anticancer mechanisms. Altogether, current results advocate potential anticancer and antiangiogenic activities of decursin and acacetin employing HUVEC and PCa models where they suppress different parameters involved in tumor growth and progression suggesting that these compounds could be potential chemopreventive agents for the prevention and management of PCa; and these conclusions warrant further pre-clinical studies for their potential clinical usefulness.