1.1 Introduction

1.1.1. Cancer epidemiology

Cancer comprises a huge burden on society in more and less economically developed countries similarly. The occurrence of cancer is increasing because of the growth and aging of the population, and in addition an increasing prevalence of well-known risk factors such as smoking, overweight, physical inactivity and altering reproductive patterns related with urbanization and economic development. Based on GLOBOCAN estimates, about 14.1 million new cancer cases and 8.2 million deaths occurred in 2012 worldwide. Over the years, the burden has shifted to less developed countries, which currently report for about 57% of cases and 65% cancer deaths worldwide.

Moreover, Other than skin cancer, prostate cancer is the most common cancer in American men. The American Cancer Society's estimates for prostate cancer in the United States for 2018 are about 164,690 new cases of prostate cancer and about 29,430 deaths from prostate cancer. About 1 man in 9 will be diagnosed with prostate cancer during his lifetime. Prostate cancer develops mainly in older men and in African-American men. About 6 cases in 10 are diagnosed in men aged 65 or older, and it is rare before age 40. The average age at the time of diagnosis is about 66 (Cancer Facts & Figures 2018). The worldwide PCa burden is expected to grow to 1.7 million new cases and 499 000 new deaths by 2030 simply due to the growth and aging of the global population (Ferlay et al., 2010).

Prostate cancer has become to a major health problem in urbanized world during the last decades of the 20th century contributing to three fourth of the registered cases across the globe (Perin, 2001). Prevalence rates of prostate malignancy vary by more than 25 fold worldwide, the maximum rates living being in Australia/New Zealand (104.2/100,000),

Western and Northern Europe, North America, mainly because the practice of PSA has been become widespread in those regions. In spite of the fact prevalence rates of prostate cancer are viewed as low in Asian and North African countries, ranging from 1 to 9/100,000 persons (Perin, 2001), demographic and epidemiological transitions in developing countries like India have exposed an increasing trend in the burden of various cancer cases including prostate cancer.

Earlier it was consideration, that incidence of prostate cancer in India is far lower as compared to the western countries but with the increased migration of rural population to the urban areas, changing life styles, increased awareness, and easy access to medical facility, more cases of prostate cancer are being selected and it is coming to the knowledge that we are not very far behind the rate from western countries.

Cancer has troubled human throughout recorded history. It is no surprise that from the dawn of history, a lot has been written about cancer. A few of the initial evidence of cancer is found amongst fossilized bone tumors, human mummies in ancient Egypt, and ancient manuscripts. Bone leftovers of mummies have exposed growths suggestive of the bone cancer, osteosarcoma. In other cases, bony skull destruction as seen in cancer of the head and neck has been found. Oldest report of cancer (although the term cancer was not used) was discovered in Egypt and dates back to approximately 1600 B.C. The Edwin Smith Papyrus, or writing, illustrates 8 cases of tumors or ulcers of the breast that were being treated by cauterization, with a tool called "the fire drill". This writing stated about the disease, "There is no treatment". The origin of the word cancer is credited to the Greek physician Hippocrates (460-370 B.C.), considered the "Father of Medicine," Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors. In Greek these words suggest a crab, in all probability connected to

the ailment on the grounds that the finger-like spreading projections from a disease brought to mind the shape of a crab. Carcinoma is the most common type of disease.

Cell growth, proliferation, apoptosis (programmed cell death), angiogenesis and differentiation are controlled by complex signal transduction pathways (regulatory circuits) (Hanahan and Weinberg, 2000). These pathways are typically activated by the binding of ligand (s) (an extracellular protein or a small molecule, such as steroid hormone) to a specific cell surface or nuclear receptor. Activation and transmission of signal frequently depends on serine or tyrosine kinases associated with the cytoplasmic portion of the receptor. Factors escalating or influencing cancer risks are varied, acting alone or in combination, usually over a period of many years. More than half of all cancers are due to variations in dietary habits, environmental exposures and life style factors including tobacco use, alcohol consumption, overweight and obesity, lack of physical activity etc. Besides, genetic predisposition and medical interventions, infectious agents such as viruses, bacteria and parasites, reproductive and hormonal factors may also account for increased cancer risk. However, most of the prevalent human cancers can, to a significant extent, be prevented and many could be avoided by a suitable choice of lifestyle and environment. Several specific causes of cancer are now identified, the mainly cause being smoking, obesity and a few oncogenic viruses, but a large proportion of global variation for common cancers such as breast, prostate, colon and rectum remain unsolved.

This dissertation was designed to identify the novel compound, which may be used as prognostic markers that discriminate indolent as opposed to aggressive form of prostate cancer. This is also very vital to understand the molecular purpose that underlies ordinary prostate increase and development, cancer initiation and development. Here, we used prostate carcinoma as a cancer cellular line model and subsequently evaluated the

anticancer and apoptotic activity of two potent flavonoids *viz.* eupalitin and cirsilineol using different phytochemicals, cytochemicals and molecular parameters along with its *in silico* study.

In the present study, structure of eupalitin and cirsilineol were characterized by using Fourier Transform Infra-Red (FTIR), proton nuclear magnetic resonance (^{1}H NMR), and carbon NMR (^{13}C NMR) spectroscopy. PC3 cells were exposed to various concentrations of eupalitin and cirsilineol investigated cell viability through MTT, the distribution of populations in cell cycle and apoptosis through different tools including ROS, morphology, caspase-3 activity, Annexin V and PI staining for apoptosis and protein expression analysis of pro-apoptotic markers BAX and $BCL_{2.}$

1.1.2. Anatomy and physiology of Prostate

In Greek the word "prostates" means "to stand before". Anatomist Heropilus named it as such because the prostate stood before the testes, as he observed it. The prostate is a part of the male reproductive system. It is located in the pelvis below the urinary bladder and in front of the rectum surrounding urethra (Moore et al., 1999). The prostate is divided into three zones: the peripheral zone, transition zone and central zone. The function of these different zones is not clear; however, in the young adult prostate gland, the peripheral zone is composed of 65-70% of the glandular tissue, the transition zone 10-15% and the central zone 20-25% (McNeal et al., 1988; McNeal et al., 1981). The luminal epithelial cells secrete fluid to nourish semen during intercourse **(**Steive et al., 1930) (Figure 1.1).

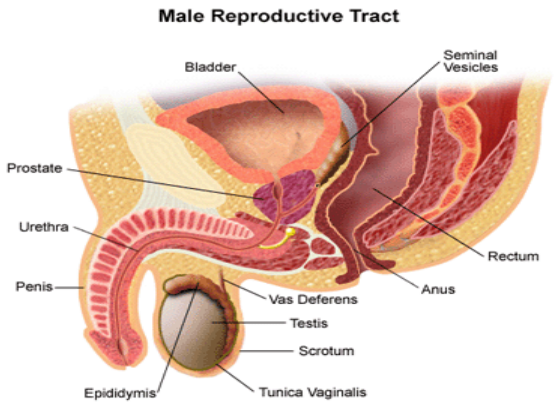

Figure 1.1 Anatomy of the male reproductive system

United States, there were an estimated 230,000 new cases of prostate cancer (PCa) and 30,000 deaths due to this cancer (Jemal et al., 2005). An estimated 48,650 German men were diagnosed with this disease and 11,839 died from PCa (www.rki.de). World health organization (WHO) announced that, 679,023 new cases and 221,002 deaths from PCa were recorded worldwide in 2002. The specific causes for PCa remain unknown (Hsing et al., 2006). The known risk factors for PCa related to age, race, diet, lifestyle, genetic predisposition (family history for PCa). The incidence rate of PCa differs with ethnicity and origin. The high incidence rate is found in USA and North Europe compared to Asian countries (Gronberg et al., 2003; Hankey et al., 1999).

1.1.3. Epidemiology and etiology of prostate gland cancer

1.1.3.1. Age

The primary risk factor for PCa is age. It is more common in men over 60 and less common in men below 45 years. Most of the tumors will be diagnosed at the age of 70 (Hankey et al., 1999). Autopsy studies have shown that approximately 30 % of men over the age of 50 and 80% of men in their 70s have microscopic evidence of prostate cancer (Breslow et al., 1977 Scardino et al., 1989). This indicates that the most men will get PCa if they live long and die due to PCa most likely.

1.1.3.2. Genetic background

A man's hereditary background contributes to his risk of developing prostate malignancy. PCa are alienated in to three diverse epidemiological forms; Familial (FPC), Hereditary (HDC) and Sporadic (SPC). The PCa patients with no family history for cancer are referred as SPC and this form constitutes about 80% of all PCa cases. SPC occurs due to the somatic mutations. FPC is defined as a clustering of PCa cases among the members of the family. HPC is a subtype of FPC which occurs through the genetic inheritance linked to PCa susceptible genetic aberrations. These two forms account for about 20% of all PCa cases recorded (Stanford et al., 2001). Men who have a brother or father with prostate cancer have twice the usual risk of developing prostate cancer (Steinberg et al., 1990).

A study on twins from north Europe suggest more risk risk for PCa in monozygotic twins compared to heterozygotic twins and reported that only 40% of PCa risk by inherited factors and 50% by other factors(Lichtenstein et al., 2000). However no single gene is responsible for prostate cancer, many genes have been implicated. Several loci such as 1q24-25, 1q36, 1q42.2-43, 8p22, 8q21, 17q-21 etc. are identified as susceptible and associated with PCa. Two genes BRAC1 and BRAC2 that are risk factors for ovarian and breast cancer have significant implications for PCa (Mazoyer et al., 2005; Struewing et al., 1997).

1.1.3.3. Diet

Dietary amounts of certain food, vitamins and minerals can contribute to prostate cancer risk. High intake of fat (which produce transfatty acids) and meat will have high risk for PCa (Giovannucci et al., 1999). Many studies have reported that dietary intake of tomato’s rich in lycopene a carotenoid with antioxidant property decrease the risk for PCa (Gann et al., 1999, Giovannucci et al., 1999). But it was challenged recently (Peters et al., 2007). Other dietary factors that may decrease PCa risk have been reported are

omega-3-fatty acids, vitamin D and E (Shirai et al., 2002), minerals zinc and selenium (Platz et al., 2001).

1.1.3.4. Alcoholism and smoking

The role of alcohol and smoking is not clear. High alcohol intake may increase the risk. Many studies have shown no correlation for PCa risk with alcoholism and smoking.

1.1.3.5. Race and ethnicity

The rate of men dying from prostate cancer has varied, depending on their race and ethnicity. In some areas of the USA, the risk of the disease is 80% higher in blacks than in Caucasians; Black men were more likely to die of prostate cancer than any other group. Chinese, Japanese (Kolonel et al., 2007) Soviet Union (Kyobutungi et al., 2004) and other Asian men have the lowest incidence of prostate cancer while men in North America and Northern Europe have the highest incidence (Quinn et al., 2002). But immigrants from these regions have more risk for PCa (Cook et al., 1999; Shimizu et al., 1991). Despite the ethnic and geographical variations in the incidence of overt disease, the incidence of latent disease has been found to be similar in all populations, suggesting that environmental factors may influence the aggressiveness of prostate cancer.

1.1.3.6. Environmental factors

Migration studies show that the incidence of prostate cancer in immigrants moving from low-risk to high-risk countries can increase with successive generations. An environmental factor that has been proposed as possibly responsible for the changes in incidence is exposure to industrial chemicals such as cadmium (Shamoun et al., 2005), lead (Telishman et al., 2007) and zinc (Novarro et al., 2007). A higher risk has also been suggested in farming possibly due to occupational exposure to chemicals used as fertilizer or in pest control (Fritschi et al., 2007; Sajid et al., 2004).

1.1.3.7. Sexual behavior

There is some implication that the risk of prostate cancer may be increased in men who become sexually active at a young age, who have multiple sexual partners or who contact a sexually transmitted disease. However, the evidence for this hypothesis is unconvincing and the finding could represent a hormonal effect.

1.1.4. Hormonal control of prostate

In the adult, prostate gland size and function is kept up through a homeostatic balance between the process of cell restoration (proliferation) and cell death (apoptosis). This balance is regulated by hormones secreted by the endocrine system, mainly androgens, of which testosterone is the major circulating form. Most testosterone (97%) circulates in the bloodstream bound to one of two proteins, either sex hormone binding globulin (SHBG) or albumin. A small percentage of testosterone (2–3%) remains unbound and it is thought to affect the glandular cells of the prostate. The enzyme 5 alpha-reductase catalyzes the conversion of testosterone to dihydroxy testosterone (DHT). DHT binds to androgen receptors (AR) within the glandular cells, then targets within the cell nucleus, specific DNA sequences known as androgen response elements, that activate cell functions, including growth and proliferation (Figure 1.2). PCa and normal prostate cells require androgens for their growth. Therefore, androgen depletion may kill PCa cells.

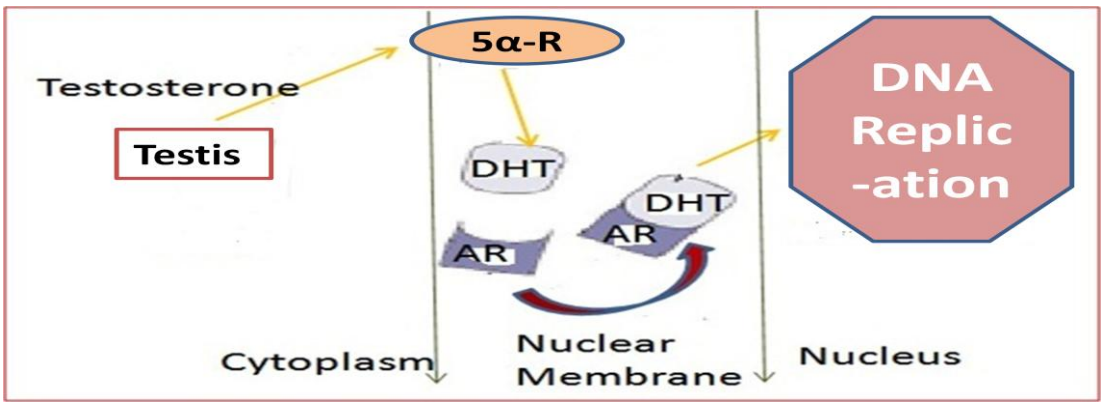

Figure 1.2 The role of androgens testosterone and DHT action in physiological function of the prostate gland

1.1.5. Prostate cancer

1.1.5.1. Prostate cancer progress

Carcinogenesis is a multi-step accumulation of genetic lesions that may eventually result in uncontrolled cellular proliferation, and/or a decrease in cell death or apoptosis. The molecular pathology of prostate cancer is not clear, but environmental, dietary, lifestyle, infection or inflammation of prostate and androgens, mainly testosterone, are thought to play a part in initiating and promoting PCa. The defects in molecular pathways that are responsible for its initiation, development and progression are still remains unclear. Various growth factors, such as epidermal growth factor and insulin growth factor and mutations in the AR may also play a role the development and/or progression of prostate cancer. At least 95% of prostate cancers are adenocarcinomas, they arise in the glandular tissue or acini derived from the epithelial cells of the prostate (Rubin et al., 2001). Most prostate cancers (60–70%) arise in the peripheral zone (PZ) of the prostate, with 5–15% arising in the CZ and the remainder developing in the TZ (Mcneal et al., 1969; Mcneal et al., 1978) (Figure 1.3).

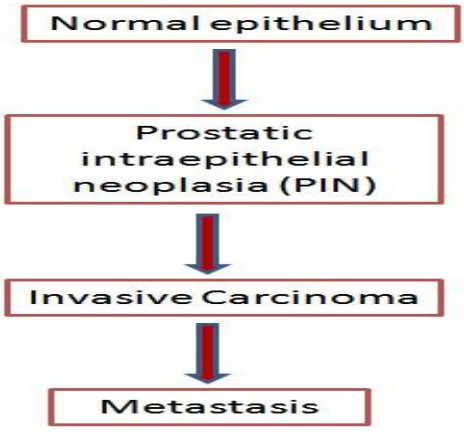

Figure 1.3 Steps involved in prostate cancer progression

1.1.5.2. Prostatic intraepithelial neoplasia

Prostatic intraepithelial neoplasia (PIN) is currently recognized as the almost certainly pre- invasive stage of PCa. Its role as the preinvasive stage of cancer was recently

confirmed (Garabedian et al., 1998; Kasper et al., 1998). PIN coexists with cancer in more than 85% of cases in PZ (Mcnael et al., 1986), the clinical importance of recognizing PIN is based on its strong association with PCa. PIN is associated with intermediate genotypic and phenotypic abnormalities between and normal and PCa. PIN is classified into two grades, low grade (PIN I) and high grade (PIN II, PIN III) (Bostwicket al., 1989; Bostwick et al., 1996) Increasing PIN associated with the loss of basal cell layer which lost completely in cancer (Lipski et al., 1996). Although there is no proof that PIN is a cancer precursor, it has been suggested that most patients with PIN will develop cancer within ten years.

1.1.5.3. Classification of prostate cancer

Grading

Gleason grading is most accepted system to describe aggressiveness of PCa histopathologically. Gleason score is assigned based on microscopic appearance of cancer tissue in figure 1.4. In grade 1, tissue resembles normal whereas, in grade 5, almost all glands are lost and tissue appears like sheet of cells. The final score is a combination of two different scores from dominant and common grades. The Gleason score is important because higher Gleasonscores are associated with worse prognosis (Gleason et al., 1974 Mellinger et al., 1967).

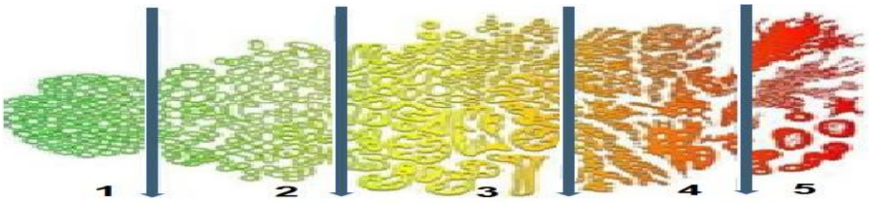

Figure 1.4 Gleason grading system to classify prostate cancer depending on its aggressiveness tumor staging

For better treatment of cancer it is necessary to know the disease stage. The most common system is the four-stage TNM system which describes primary tumor (T), tumor

in lymph nodes (N) and metastasis (M). In the TNM system, clinical T1 and T2 cancers are found within the prostate, while T3 and T4 cancers have spread elsewhere in the body (Wittekind et al., 2002).

1.1.5.4. Diagnosis

Prostate cancer is generally slow-growing. In several cases the disease did not reach a stage where it becomes clinically significant. Screening for prostate cancer involves the digital rectal examination (DRE) and the prostate specific antigen (PSA) blood test. Either of these tests alone cannot detect cancer. Both tests together are valuable methods in detecting this life threatening cancer. Consequently, a pathological PSA value and/or DRE necessitate further diagnostic procedures, such as the trans-rectal ultrasound (TRUS) examination with multiple biopsies from the prostate. Latest research investigations suggest that sampling of increased biopsies improves the PCa detection (DeMarzo et al., 2003**;** Taylor et al., 2002). Patients with high PSA levels (> 4 ng/ml) and negative biopsies require annual testing of PSA and further biopsies to exclude PCa.

1.1.5.5. Biomarkers for early diagnosis

Since the introduction of PSA, the detected incidence has increased dramatically (Kramer et al,. 1993). However, PSA is neither tissue nor gender specific and small amounts are also detected in the endometrial, breast, adrenal and renal tumors. Moreover, PSA can be secreted from benign as well as malignant cells of the prostate (Martinez et al., 2002; Polascik et al., 1999). Therefore, the serum PSA value correlates closely with both, BPH and PCa. Most men will have PSA level between 4-10 ng/ml (Woolf, 1995) which demands better tools for early diagnosis of cancer in these patients to save life.

Several markers for PCa have been reported but only a few potential markers have been identified such as Alpha-methylacyl-CoA racemase (AMACR) (Rubin et al., 2002;

Jiang et al., 2002). The only test which could completely affirm the diagnosis of prostate cancer is a biopsy, the taking away of tiny pieces of prostate tissue for microscopic examination. Although some parameters including measurement of free and total PSA value (Stenman et al 1997; Catalona et al., 1998). Human glandular kallikrein-2 (Parekh et al., 2007; Stephan et al., 2006) and insulin growth factor-1 in blood serum, immunohistochemistry of Ki-67 proliferation index (Feneley et al., 1996; Weinstein, 1998) and CD44 have been employed; a reliable marker to decide whether further biopsies are indicated has not yet been established. Consequently, it is necessary to develop novel diagnostic methods to improve early detection of PCa. Recently a new blood test for early prostate cancer antigen-2 (EPCA-2) was reported for PCa diagnosis and staging (Hansel et al., 2007; Paul et al., 2005).

1.1.5.6. Prostate cancer chemoprevention

Prevention of prostate cancer has come to light as one technique to decrease the public issue trouble of prostate cancer. Diverse approaches are applied in prevention of prostate cancer including both number one and secondary prevention method. Primary prevention is the technique of stopping the disorder before it arises, although the preferred preventive strategy with highly defined and amendable risk factors are much less well described in prostate cancer. Therefore, studies into the mechanism of initiation of prostate carcinoma are vital to facilitate for the recognition of the specific molecular occasions and risk factors which are involved in prostate cancer transformation before primary prevention methods.

On the other hand, secondary prevention refers to intrusions that prevent or decrease the development of a disease at an early stage, thereby restraining incapacity as soon as the disease is identified. For this reason, even as the simple incidence of the ailment may not

be altered via secondary prevention measures, development to clinically apparent disease may be reduced.

1.1.5.7. Treatment

The prognosis and treatment options depend on tumor stage, patient's health, age and/or weather cancer diagnosed early. For tumors that are localized inside the prostate, radiation treatment, watchful waiting and a surgery called radical prostatectomy are regular treatment alternatives. The tumors that spread the capsule prostate can't be cured with either radiation or surgery. They can be treated with hormone ablation (high level of androgen therapy).

1.1.6. Diet and Cancer

1.1.6.1 Background

The role of dietary affects in cancer has been studied for the reason that 1940's when Tannenbaum and others demonstrated the tumor promoting effects of various nutritional constituents, mainly dietary fats, in animal models (Silverstone et al., 1950; Boutwell et al,. 1949; Tannenbaum et al., 1949). Dietary elements were predicted to contribute up to 30% to cancer development and mortality, even though this determine counseled by means of doll et al is relatively speculative (Doll and Peto 1981). Whilst the animal experiments of the early to mid 1900's set the scene for diet-cancer hypotheses, the emergence of sturdy epidemiological observations within the 1960's and 1970's linking nutritional behavior with cancer prevalence international-huge reignited interest inside the area of diet and cancer (Armstrong and Doll, 1975; Lea, 1966). Armstrong and Doll's study of correlations among nutritional habits and cancer incidence and mortality in 32 nations likely obtained the best interest. The strongest institutions in this study have been meat consumption with colon cancer and consumption with breast and uterine cancer, even though weaker correlation of fats and prostate and other cancers changed into also

demonstrated (Armstrong and Doll, 1975). Many diet regimen-cancer hypotheses were extrapolated from those epidemiologic observations. Because the most powerful institutions had been from international geographical correlations in which confounding variables are incompletely accounted for, and estimations of dietary intake are often flawed, some argue that conclusions and nutritional suggestions were inappropriately reached based totally in simple terms on indirect epidemiologic proof (Enstrom, 1981). Enstrom additionally questions the selective interpretation of facts from migration research in which despite the fact that an expanded risk of breast and colon cancer is located in Jap migrants to the USA, the total cancers diet for Japanese Americans individuals is unchanged and normal mortality is simply lower than that of Jap natives. The mission inside the area of nutrition and cancer is that testing these hypotheses within the placing of randomized controlled trials is frequently tough due to terrible patient compliance and the significant expense required for such great trials. Most research in the area in the last two decades has targeted on determining diet and cancer associations by using properly designed case-control and prospective cohort studies. The fashion has been that case-control studies have a tendency to expose a correlation among a nutritional element and cancer risk that isn't always evident whilst tested by way of prospective cohort studies. Part of the problem with case- control research investigating an association of diet with cancer is the sturdy impact of both considers and choice bias on this form of has a look at layout (Willett, 2005). Don't forget of diet may be biased by means of the prognosis of cancer wherein case sufferers can also unduly associate particular bad dietary habits with their diagnosis (Giovannucci et al., 1993). Case and control choice in this research is often biased considering the fact that although cancer sufferers have a high diploma of participation, control participation is typically low. Moreover, control subjects who do take part are commonly more health conscious and

have healthier nutritional behavior, therefore accentuating the inverse affiliation of healthful dietary habits with most cancers in case-control studies (Willett, 2005). A first-rate complicating aspect in our interpretations of epidemiologic research of diet regimen and prostate cancer is the confounding role of prostate specific antigen screening on observes consequences (Giovannucci, 2007). That is highlighted by means of the disparity in results of epidemiologic studies of lycopene and prostate cancer occurrence conducted in the pre- and publish-PSA era. A shielding impact of lycopene became validated in studies pre-PSA screening whilst the effects of more current research have been negative. Giovannucci has proposed that this trend may be accounted for by the reduced probability of detecting a sizable impact of lycopene in populations which might be PSA screened (Giovannucci, 2007). The inverse affiliation of lycopene and prostate most cancers occurrence in pre-prostate cancer antigen generation research became most powerful for metastatic disorder. Seeing that most PSA diagnosed cancer are early and organ-limited, the resulting dilution of the variety of advanced cancers might also suggest that publish-prostate cancer antigen technology studies fail to expose an effect on superior disorder (Giovannucci, 2007). This is supported by means of findings of prospective research together with the health experts examine that spanned both the pre- and publish-PSA eras. Different sources of inaccuracy and bias in the design of nutritional research were within the inconsistencies related to amassing correct records on dietary habits. Willett et al had been leaders in designing tested diet plan frequency questionnaires (Willett and Hu, 2006; Willett and Hu, 2007; Willett et al., 2007). Although their diet regimen questionnaires have been widely followed, validation of this device by way of weighing player diets for one week and comparing to the questionnaire established correlation of 0.5-0.6 (Willett et al., 2007). The considerable variability in nutritional consumption on a day-to-day foundation makes it extremely difficult to as it should be

estimate dietary intake. Despite the fact that the application of meals frequency questionnaires has been wondered, their efficacy has been confirmed in research highlighting the consequences of diet in cardiovascular disorder (Hu and Willett, 2002). Consequently, loss of positive research the usage of these questionnaires in cancer studies isn't always solely due to the inaccuracy of the questionnaire, but moderately could be due to issues related to cancer such as difficulty of causation and latency. Whilst estimating the consumption of unique nutrients or micronutrients, food frequency questionnaires can be pretty misleading owing to the complexity of micro- and macro-nutrients inside the food plan, and the enormous effect of food processing and cooking on the availability of these nutrients. When examining a heterogeneous organization of micronutrients along with flavonoids or carotenoids, a similarly supply of bias can be that the full micronutrient content of specific meals may not were determined. As an example, whilst the nutrition a content of many foods is thought, the whole carotenoid tiers for those meals are not acknowledged. For that reason, even as β-carotene won't correlate with a particular sickness, the identical conclusions cannot be reached for total carotenoid intake. The USA department of agriculture flavonoid content has attempted to address this deficiency in our knowledge for flavonoid content material of various meals; however this database is constrained to only some flavonoids.

Over the past many years a number of nutritional institutions with cancer risk have received credibility, whilst at the identical time many hypotheses were refuted. Development on this area has been slow to supply specific guidelines for dietary adjustments for improving cancer risk not least for the difficulties mentioned above. Another cause, in line with Giovannucci, for the sluggish progress within the area of nutrients and cancer, in comparison to the examiner of dietary factors in cardiovascular ailment has been the absence of intermediate biomarkers (which includes blood strain or

lipid profile in cardiovascular ailment) this means that that most cancers research must depend on lengthy-time period behind schedule final results measures (McCullough and Giovannucci, 2004). In end, the studies analyzing nutritional institutions with most cancers are especially inconsistent. The type of approaches (case-control, cohort etc) and the variations in adjustment for confounding variables makes it hard to reach company conclusions on dietary associations with cancer improvement. While retrospective researches have shown correlations of dietary elements with most cancers prevalence, prospective cohort researches have in many cases failed to aid those effects. There is an effect on of prostate specific antigen screening on the outcome of recent epidemiologic studies is any other vital element including to the issue of demonstrating any nutritional associations with prostate most cancers risk. Beneath, i can cope with the maximum pertinent reviews of the function of nutritional elements and cancer risk, with a focus on dietary associations with prostate cancer.

1.1.6.2. Role of Macronutrients in Cancer

Probably the most intensely studied of all dietary institutions is that of fats and cancer. The availability of international age-adjusted mortality fees in the 1960's for numerous malignancies paved the manner for analytic epidemiologic studies of nutritional behavior and cancer that highlighted the high quality affiliation of dietary fat consumption and cancer (Mccullough and Giovannucci, 2004). Epidemiologic studies correlated the excessive consumption of fats in advanced international locations with increased prevalence of colon, breast, and ovary, endometrial and prostate. Epidemiologic research of this nature fail to don't forget foremost confounding variables that would impact disease hazard, including age of menarche or parity in breast cancer risk (Willett, 2001). Information from case-control studies of fat intake and breast cancer has been inconsistent, with research demonstrating both fine and bad correlations. Several potential

cohort researches have failed to reveal an affiliation with fats intake and breast cancer, as did the girl's fitness initiative randomized control trial. The consensus, consequently, is that there's no affiliation between fat consumption and breast cancer risk (Kushi and Giovannucci, 2002). Most research of fat consumption and colon most cancers risk further have shown no correlation, excluding the nurse's health have a look at that demonstrated a statistically tremendous increased risk (relative risk of 1.89) of colon cancer among women in the maximum and lowest quintiles of fats consumption (Willett et al., 1990). Maximum analytic epidemiologic research of prostate cancer has failed to show a correlation with fat intake. Early case-control studies that did display a sizable correlation failed to manage for general energy consumption (Kolonel and Nomura, 1999). Interestingly the form of fats may be vital, with alpha-linolenic acid specifically associated with improved cancer risk in numerous research. Accordingly, while early population research have correlated fat consumption with cancers of the breast, colon, and prostate, prospective research have in large part failed to confirm those institutions possibly reflecting the shortage of appropriate adjustment for confounding variables in populace studies (Moyad, 2002). A problem springing up from the evaluation of nutritional research of fat and cancer risk is the inverse correlation of fat intake with carbohydrate intake. Therefore, it could be hard to decide the volume to which effects of research inspecting changes in fat intake are attributable to fat or carbohydrate versions (Key and Spencer, 2007). Carbohydrate consumption in itself has been connected to cancer risk. In a assessment of 21 case-control studies of sugar and colorectal cancer, 7 tested superb correlation among sugar consumption and colorectal cancer risk (Burley, 1997). Prospective cohort studies have failed to show huge correlations of sugar consumption or glycemic load and colon cancer risk, even though effective traits are apparent (Key and Spencer, 2007). Further for breast cancer, all prospective studies

however one (Holmes et al., 2004) failed to expose a good sized correlation with carbohydrate intake. Studies of the effect of carbohydrates in prostate cancer had been mainly constrained to animal experiments that have shown the inhibition of prostate cancer xenograft growth in mice fed low carbohydrate diets in comparison to high carbohydrate diets (Freedl et al., 2007; Hsing et al., 2001). This effect became associated with a reduction in tiers of insulin, IGF-1 and its downstream target akt. Insulin and IGF-1 are both growth hormones which have been associated with increased risk of prostate cancer (Renehan et al., 2004). A vulnerable however tremendous superb correlation of fasting serum glucose tiers turned into demonstrated in a huge Icelandic cohort circuitously suggesting a probable dating among carbohydrates in the food regimen and prostate cancer danger (Tulinius et al., 2007). In summary, whilst outcomes of animal experiments are promising, the affiliation of carbohydrates with colorectal and breast cancer is minimal based on analytic epidemiologic studies, and the affiliation with prostate cancer is but to be safely investigated in human beings. The effect of dietary protein consumption on tumor genesis has been studied for over 50 years. Animal experiments have verified a reduction in onset of numerous varieties of malignancy in animals fed low protein (casein) compared to excessive protein diets (Campbell, 2007).Verification of these observations in the human placing has been much less extensively studied than other macronutrients. A small case-control have a look at demonstrated that excessive protein intake changed into related to increased stages of growth elements including IGF-1 (Iontana et al., 2006). An in depth report by using the world cancer research fund (WCRF) did not include protein in its suggestions of dietary changes primarily based on substantial evaluation of to be had literature (seventy one). The intakes of meat and dairy products, each main sources of nutritional protein had been substantially studied; but, given that these additionally represent major resources of

different vitamins inclusive of saturated fats, correlations with cancer risk cannot be directly attributed to protein in that research. Consequently, further research is wanted to absolutely decide the consequences of protein intake on carcinogenesis. The proof for meat is mainly robust for colorectal cancer, with a latest meta- evaluation of numerous cohort studies by way of the WCRF concluding that 'pork is a convincing purpose of colorectal cancer' (seventy one). This conclusion is debated by using others who web site the absence of correlation or certainly the inverse affiliation of meat and colon most cancers risk in several big cohort research (Hill, 2002). The principal research demonstrating multiplied colorectal cancer risk with meat are the nurses' and health expert research in which a widespread danger became associated with very excessive meat consumption >140g in line with day. As a result, while there are conflicting reports in the literature, the consensus is that meat intake of >140g in step with day is adverse (Giovannucci et al., 1994). Meta-analyses of cohort studies examining meat and breast cancer have proven a slight or no affiliation (Boyd et al., 2003; Missmer et al., 2002). Numerous case-control and cohort research have shown sizable fantastic correlation of meat consumption with prostate cancer (Kolonel, 2001). Consequently, the evidence for meat as an etiological element in most cancers appears to be strongest for colorectal and prostate cancer.

The WCRF take a look at panel additionally concluded that milk and dairy products likely have a protective function in colon cancer, at the same time as the effects of cohort research in prostate cancer are inconsistent (seventy one). The emergence of obesity as a public fitness problem was highlighted as early as the early 1900's whilst big studies subsidized by using the yank existence insurance enterprise confirmed a effective affiliation between untimely mortality and body construct (Rothstein, 2006). A century later and the affiliation of weight problems to mortality from all causes and most cancers

remains highlighted in epidemiologic research (Manson et al., 1985). The International Association for Cancer Research (IARC) performed an in depth evaluation of the evidence linking weight problems with cancer in 2002. This record counseled that the evidence helping the affiliation of obesity to cancer was finest for colorectum, breast, endometrium, kidney and esophagus. The evidence for an affiliation of obesity and prostate cancer turned into greater inconsistent. That is notwithstanding the final results of the most important examine examining obesity and prostate cancer chance in a Norwegian cohort of 950,000 men with 33,000 incident prostate cancer cases that confirmed a good sized affiliation of weight problems with prevalence of prostate cancer in men aged 50-59 (RR 1.59) (Engeland., 2003). Prospective cohort studies posted after 2002 continued to aid this conclusion for both prostate and non-prostate cancers (Zhang et al., 2005). Apparently, even as the statistics suggest a susceptible association of obesity with occurrence of prostate cancer, the affiliation is truly stronger for mortality from prostate cancer. The American cancer society cancer prevention study i and ii enrolled over 900,000 participants and validated a full-size fantastic association of obesity and prostate cancer mortality (RR 1.27) (Rodriguez et al., 2001). This affiliation became also showed in a potential cohort study of 135,000 Swedish creation employees (Andersson et al., 1997). This supports the notion that obesity has a position in progression in preference to initiation of prostate cancer. Consequently, the evidence suggests that caloric intake, obesity and macronutrient intake are all essential factors in the pathogenesis of prostate cancer.

1.1.6.3 Effect of Micronutrients and photochemical in Cancer Prevention

Micronutrients, vitamins, minerals and trace factors are important elements of diets and are wished in small quantities. Photochemical are non-critical bioactive components derived from plant products. Hundreds of photochemical exist in nature, and many had

been evolved into pharmaceutical dealers in use today, while many greater are presently below investigation. Examples of phytochemicals consist of flavonoids, stilbenes (e.g. Resveretrol), and isothiocyanates amongst many others. Many of these lessons of compounds were shown to possess anti-oxidant, anti inflammatory, anti-microbial and anti- cancer effects. Culmination and greens, tea, and medicinal herbs are all wealthy sources of photochemical. End result and vegetables had been used for medicinal capabilities for millennia. The numerous case-control and cohort studies examining the relationship of fruit and vegetable consumption with cancer chance have these days been reviewed in a file by way of the WCRF (seventy one). The authors tested proof for non-starchy greens and fruits separately. The evidence for non-starchy vegetable consumption appears to be strongest for mouth, pharynx and larynx cancers where a large frame of proof has proven a dose-response effect, while the proof in lung, belly, colorectal, and ovarian cancers was restricted. The evidence for the cancer preventive effect of fruit consumption was strongest for lung cancer, weaker for esophageal and mouth, pharynx and larynx cancer, and restricted proof existed for different cancer. Alas, the WCRF document did now not assessment the association of fruit or vegetable intake on prostate or breast cancer.

Numerous case-control and cohort research have tested the hyperlink among fruit and vegetable intake and prostate cancer (Severson et al., 1989; Tzonou et al., 1999). Early case-manipulate studies found out no association of fruit and vegetable consumption and prostate cancer risk (lee et al., 1998). Larger cohort has a look at have lately corroborated this end (Kirsh et al., 2003). The European prospective investigation into cancer and nutrition (EPIC), a cohort of over 130 000 men from seven European countries, stated no association. In a current prospective observe of diet program and prostate cancer in nearly 30,000 guys, higher consumption of veggies changed into associated with a large

reduction only in high grade PCa (RR0.41), especially cruciferous greens together with broccoli (RR 0.55) and cauliflower (RR 0.forty eight) (Kirsh et al., 2003). However, other potential cohort research have failed to show a large inverse association of cruciferous vegetable intake and prostate cancer risk (Lee et al., 2003).tomato merchandise have validated shielding effects on PCa development, despite the fact that the information is rather conflicting. Tomatoes are excessive in lycopene, a potent free radical scavenger of the carotenoid circle of relatives. In a latest meta-analysis, high as opposed to low intake of tomatoes become related to a 10% to 20% discount in PCa risk (Etminan et al., 2004). The affiliation become stronger for cooked versus raw tomato products. In a prospective cohort take a look at, Giovannucci et al suggested that tomato sauce consumption turned into related to a 35% reduced chance of incident advanced PCa (Giovannucci et al., 2002). Several research have no longer proven an association of lycopene with PCA risk and it's been proposed that the terrible outcomes of 3 of those studies can also were because of the typically low intake of tomato merchandise inside the take a look at populations (Chan et al., 20005). The lately carried out U.S. food and Drug Administration's review t of the evidence for an association between tomatoes, lycopene and prostate cancer mentioned very little proof helping an affiliation (Kavanaugh et al., 2007). The effect of prostate cancer antigen screening on the outcome of lycopene research become proposed via Giovannucci to account for the dearth of an association of lycopene and prostate cancer in recent epidemiologic research, as was discussed earlier (Giovannucci, et al., 2007). Other micronutrients with postulated consequences in prostate cancer consist of vitamin E and selenium. The initial proof for vitamin E emerged from the alpha-tocopherol beta-carotene cancer prevention trial that stated statistically large discounts in PCa incidence and mortality in men randomly assigned to alpha-tocopherol versus placebo (Heinonen et al., 1998). Further, gamma-tocopherol has

additionally established an inverse association with PCa risk in epidemiologic research (Helzlsouer et al., 2000; Huang et al., 2003). Numerous researches however have failed to expose any association of alpha or gamma tocopherol consumption and PCa (Nomura et al., 1997). The evidence for the impact of selenium is stronger, with some of massive potential cohort research and a randomized controlled trial demonstrating an inverse association of selenium intake and prostate cancer (Yoshizawa et al., 1998). The chemopreventive outcomes of selenium and vitamin E are presently being tested in a randomized managed trial (pick) of 32,four hundred men, the consequences of that are expected in 2013 (Klein et al 2001).

In vitro, animal and epidemiological research has pointed to the chemopreventive function of vitamin D in cancer. First recommend the vitamin D hypothesis for PCa, postulating that various epidemiologic risk factors for PCa (growing age, black race, northern range) might be explained with the aid of relative vitamin D deficiency (Schwartz and Hulka, 1990). Supporting this speculation are epidemiological studies that have tested that solar ultraviolet-B radiation, the maximum critical source of vitamin D, is associated with reduced risk of several malignancies along with prostate cancer (Grant, 2002; Colli and Furnish, 2008). Furthermore, hanging discounts in cancer mortality had been verified for cancers diagnosed in summer months when vitamin D, stages are highest, compared to winter (Porojnicu et al., 2007). The discovery that many cell types, along with prostate epithelial cells express vitamin D receptors, lends similarly motive to this hypothesis (Peehl et al., 1994). Prostate epithelial cells also explicit the vitamin D metabolizing enzyme 1- alpha-hydroxylase which converts circulating 25-hyroxyvitamin d (25-d) into the more potent 1, 25 dihydroxy vitamin D (1, 25-d) (Schwartz et al., 1998). 1, 25-d has been proven to sell differentiation and inhibit proliferation and invasiveness in vitro (Skowronski et al., 1993; Schwartz et al., 1997). Epidemiological studies of the

affiliation of PCa with serum vitamin D tiers were conflicting. Several small serum based totally case-control studies have proven no distinction in serum diet D (each 25-oh and 1,25-oh vitamin d) ranges among cases and controls (Jacobs et al., 2004; Platz et al., 2004), whilst others have confirmed a defensive impact of better tiers of 25-oh vitamin D in PCa (Ahonen et al., 2001). Nutritional intake studies of vitamin D consumption or supplementation have not supported the function of vitamin D in prostate cancer threat (Kristal et al., 2002). This is in assessment to colorectal cancer in which nutritional vitamin D intake is greater strongly associated with decreased cancer threat (Provide and Garland, 2004). It has been postulated that decrease degrees of 1-alpha hydroxylase in prostate cancer cells as compared to colon cancer cells can also account for the disparity within the effect of vitamin D in prostate and colon cancers (Hsu et al., 2001). Calcium dose has been revealed to be associated with prostate cancer chance. Previous look at has shown the positive members of the family among calcium intake and PCa chance, predominantly for advanced sickness and at higher intake of calcium (Rodriguez et al., 2003). In a previous document of eleven case-control studies, between calcium consumption and PCa risk , the blended odds ratio changed into determined to be 1.68 (95% ci = 1.34-2.12) (qin et al., 2004). A meta- analysis of approaching cohort research confirmed a relative risk of total prostate cancer of (95% CI 1.00, 1.22; P=0.047) in the highest vs. The lowest intake agencies of dairy merchandise 1.11 and 1.39 (95% CI 1.09, 1.77; P=0.018) within the highest vs. Lowest consumption corporations of calcium doses (Gao et al., 2005). The hypothesis that improved calcium consumption increases PCa risk by using distinctive feature of lowering 1,25-oh vitamin D tiers is not supported through studies demonstrating that big variations in calcium intake are associated with small adjustments in 1,25-oh diet d stages (Bonjour et al., 2007). As a consequence, the effect

of calcium on prostate cancer progression is most likely unrelated to the vitamin D pathway.

The special molecular outcomes of vitamin D, calcium and other micronutrients together with selenium, vitamin E, and lycopene are beyond the scope of this thesis and have been reviewed someplace else (Guns and Cowell, 2005). These theses will recognition instead at the mobile and molecular results of every other critical elegance of antioxidant phytochemicals- the flavonoids. Flavonoids are fine known as the red, blue and purple pigments of flowering plants (because of the anthocyanidin sub-organization) despite the fact that the purple pigment of a few end result may be due to carotenoids including lycopene (Timberlake and Henry, 1986). Those pigments and the yellow pigments of flavones and flavanols are also chargeable for the autumn leaves color (Winkel, 2001). Due to the importance of color in pollinator enchantment, flavonoids have a vital position in plant replica. Flavonoids in plant life also serve to protect from ultraviolet (UV) light owing to their high UV absorbance coefficients, and it's been counseled that this belongings of flavonoids was critical within the evolution of aquatic vegetation to a terrestrial lifestyles. Flavonoids also own other essential features in protection towards microorganisms and germination of pollen (Taylor and Frotewold, 2005; Reutter, 2005).Biosynthesis of flavonoids in plants is via a series of enzymatic steps beginning with the aromatic amino acid phenylalanine and acetate (Winkel, 2001). The flavonoid pathway is regulated in plants consistent with environmental stimuli that cause changes in transcription of diverse enzymes inside the pathway (Winkel, 2007). This results inside the generation of flavonoids with unique characteristic in step with the survival needs of the plant. The flavonoid pathway is one of the most virtually described of all secondary metabolism pathways in vegetation. This has been in part attributed to the brilliant colours of flavonoids that significantly facilitate the monitoring of experiments in

flavonoid biology. An interesting possibility that has emerged with the know-how of flavonoid biochemical pathways has been the utility of biotechnology methods to generate plants or even bacteria which are genetically engineered to provide excessive stages of a selected flavonoid for human use (Ververidis et al., 2007). Every institution of flavonoids possesses precise chemical nature and has a specific distribution in flowers. Anthocyanins (glycosylated anthocyanidins) and proanthocyanidins (polymers that produce anthocyanidins when hydrolyzed) broadly speaking offer shade to flowering flowers and fruit, and are therefore discovered in excessive concentrations within the pores and skin of pink grapes, purple wine.

1.1.7. Role of flavonoids in prostate cancer chemoprevention

1.1.7.1. Definition, Classification & Chemistry

Phenolics are the maximum ubiquitous class of plant chemical substances, and encompass compounds with a hydroxyl organization (-OH) attached to an fragrant hydrocarbon. Flavonoids are a unique magnificence of polyphenolic plant secondary metabolites. The term ‘flavonoids’ incorporate a diverse variety of compounds all of that are based at the C6-C3-C6 (phenylbenzopyran) carbon skeleton. Flavonoids are placed within the cells or at the floor of diverse plant organs and have numerous capabilities in plants (Marais et al., 2006).The underlying basic structure of all flavonoids consists of 2 fragrant rings, the ‘a’ and‘b’ rings linked with the aid of a three-carbon bridge. For most flavonoids, the three-carbon bridge combines with oxygen and the ‘a’ ring to form a third important ring structure, the ‘c’ ring. The central ring shape is known as a chromane ring and is a shared shape of flavonoids and tocopherols. Chalcones, a sub-elegance of flavonoids, lack the critical ‘c’ ring. Flavonoids are categorized into several groups based on particular structural capabilities: the foremost flavonoids, isoflavonoids, and neoflavonoids (Marais et al., 2006).The predominant flavonoids, isoflavonoids and neoflavonoids are isomers

that fluctuate in step with the placement of the fragrant B-ring moiety .different variations of the flavonoid systems include the chalcone and aurone flavonoid families which can be called minor flavonoids. The group of important flavonoids is similarly subcategorized based on variations in structure of the critical chromane ring. Distinct stages of saturation of the chromane ring arise with flavanones and flavanols having no carbon-carbon double bonds inside the middle ring; flavones having one c-c double bond; and anthocyanidins possessing two C-C double bonds. Flavanones and flavanols are differentiated based totally at the presence of a carbonyl (C=O) organization at function four at the chromane ring of flavanones that's absent in flavanols. This C=O sensible group is likewise seen in flavones.flavonols are very just like flavones differing only by means of the addition of a hydroxyl at the 3- role on the principal ring. Flavanols generally have a hydroxyl on the three-role and are consequently termed flavan-3-ols, assisting to keep away from confusion with the similar sounding flavonols. Anthocyanidins are particular in that the oxygen atom of the chromane ring has a effective charg.up till the middle of the 20th century, flavonoids had been believed to be waste merchandise of plant primary metabolism, a belief that turned into quickly abandoned based totally on studies demonstrating the myriad capabilities of flavonoids in plant survival (Øyvind and Andersen, 2006).we now understand the complicated metabolic pathways used to synthesize flavonoids in vegetation- pathways which have evolved over millennia to provide a survival gain to plants. Vegetation, absolutely do no longer have the posh of mobility and have had to evolve complicated chemical mechanisms so that you can shield themselves from numerous insults (inclusive of uv radiation) and so that it will attract those on the way to help with their replica (bees, birds) (Prasain and Barnes, 2007). Certainly, the complexity of the plant genome, which exceeds that of human beings, is

assumed to have advanced to keep those immensely complex pathways of synthesis of novel chemicals (Prasain and Barnes, 2007).

Flavonoids are first-rate known as the red, blue and red pigments of flowering flora (due to the anthocyanidin sub-group) despite the fact that the purple pigment of a few culminations can be because of carotenoids which includes lycopene (Timberlake and Henry, 1986). Those pigments and the yellow pigments of flavones and flavanols are also liable for the fall leaves color (Winke, 2001). Due to the significance of colour in pollinator enchantment, flavonoids have an essential role in plant duplicate. Flavonoids in flowers also serve to defend from ultraviolet (UV) light due to their high UV absorbance coefficients, and it's been recommended that this asset of flavonoids modified into vital in the evolution of aquatic plant life to a terrestrial life (Øyvind and Andersen, 2006). Flavonoids additionally own different important functions in protection towards microorganisms and germination of pollen (Taylor and Grotewold, 2005).

Biosynthesis of flavonoids in flowers is via a series of enzymatic steps beginning with the aromatic amino acid phenylalanine and acetate (Winkel, 2001). The flavonoid pathway is regulated in flora consistent with environmental stimuli that reason changes in transcription of various enzymes in the pathway (Winkel, 2002). These outcomes within the generation of flavonoids with particular function in step with the survival needs of the plant. The flavonoid pathway is one of the most honestly described of all secondary metabolism pathways in plant life. This has been partly attributed to the bright colorations of flavonoids that significantly facilitate the tracking of experiments in flavonoid biology. An fascinating possibility that has emerged with the know-how of flavonoid biochemical pathways has been the utility of biotechnology methods to generate flowers or even micro organism which can be genetically engineered to supply high levels of a specific flavonoid for human use (Ververidis et al., 2007). Every institution of flavonoids

possesses specific chemical houses and has a specific distribution in flowers. Anthocyanins (glycosylated anthocyanidins) and proanthocyanidins (polymers that produce anthocyanidins when hydrolyzed) mostly offer coloration to flowering vegetation and fruit, and are consequently found in excessive concentrations in the pores and skin of pink grapes, red wine and berries. Flavan-3-ols, along with catechin, epicatechin gallate are colorless and are determined in high concentrations in green tea. Isoflavones are most effective located in legumes (e.g. Soy) and are therefore fed on in high quantities in regions of the world with excessive soy consumption. Flavanones are located in high levels in citrus fruits, whilst flavones are present in green leafy spices which include parsley, and flavonols are ubiquitous and observed in maximum culmination and vegetables fed on inside the human diet regimen (Harnly et al., 2006). Over 9000 flavonoids had been observed in nature (a hundred and sixty). The variety of theoretically feasible flavonoids is even greater. This big variety is because of the huge variety of viable substitutions (hydroxyl, methoxyl, methyl, isoprenyl, benzyl and many others) on the diverse positions on the flavonoid carbon framework. Moreover, every hydroxyl institution and some carbons may be substituted by using a variety of various sugars, which can be substituted themselves by way of numerous organic and aliphatic acids (Williams and Grayer, 2004). Due to their common presence in plant life they constitute a critical a part of the human diet and estimates of every day consumption range from 50-1000mg overall flavonoid intake daily (Hertog et al., 1993). The huge variations in expected flavonoid consumption are partly due to the estimation of various types of flavonoids through distinct research. Most flavonoids in nature are glycosylated, and therefore, the studies estimating flavonoid glycoside consumption quote flavonoid consumption at numerous folds better than studies measuring aglycone consumption. Regardless of the estimation, the intake of flavonoids even inside the western diet that is

low in culmination and greens greatly surpasses the intake of other photochemical inclusive of vitamin E and β-carotene (Hertog et al., 1993). Due to the large human consumption, it's far vital that we take a look at carefully the safety and underlying outcomes of flavonoids on human health and disorder. Interest in the fitness advantages of flavonoids stemmed from early studies in 1936 with the aid of the Hungarian scientist Szent-Gyorgyi, who also incidentally discovered vitamin C. He isolated a substance, which he known as citrin, from lemons that restored weakened capillaries to everyday. This effect became neglected whilst vitamin C alone was administered. Citrin changed into later proven to be composed of the flavonoids hesperidin and eriodictyol. Even though citrine turned into also known as vitamin P, this name became dropped within the Nineteen Fifties after it turned into concluded that flavonoids had not suit the stern definition of a diet (Harborne, 1986). Despite not qualifying as vitamins, flavonoids have been shown in the last 72 years since Szent-Gyorgyi's initial discovery to affect various aspects of human health not limited to their beneficial effects on capillary wall integrity.

The variety of outcomes of flavonoids on mammalian biological structures is not anything quick of superb. The cell techniques modulated by flavonoids *in vitro* range within the masses. Some issues emerge from the numerous hundreds of research on this area. First off, the consequences of flavonoids at the same time as remarkable *in vitro* aren't conclusively translated in the *in vivo* scenario. This will be accounted for by using a number of of factors which include poor absorption of flavonoids, tremendous metabolism, and the complexity of the milieu *in vivo*, that's poorly replicated with *in vitro* experiments. Secondly, the literature factors to the significance of flavonoid shape of their biological results, such that positive systems (e.g. Flavonols) have drastically greater organic effect than others (e.g. Flavanones). One cause of this latter effect may be the extra planar structure of flavonols (due to the double bond within the c-ring) that makes

these molecules much more likely to engage with active websites on enzymes. Many different mechanisms are probably to account for variations among character flavonoids. Such generalizations that emerge from the literature allow us to position flavonoid studies in attitude. As a result, we want to be aware that during vitro flavonoid effect must no longer be taken to immediately mean *in vivo* interest and that the outcomes of magnificence of flavonoid do no longer always observe to all flavonoid types.

1.1.7.2. The Epidemiology of Flavonoids in Relation to Cancer

A vast epidemiologic literature helps the beneficial health results of flavonoid intake. The principle regions of interest were the anti-cancer and cardio-defensive consequences of flavonoids. Prostate cancer has received special attention in this regard attributable to observations of dramatic differences in mortality of prostate cancer among populations eating excessive (China) and low (North the United states/Europe) ranges of flavonoids. Epidemiological studies, including numerous case-control and cohort research have widely supported this hypothesis. Simplest 2 out of 7 case- control studies failed to expose a protective impact of flavonoid intake and prostate cancer risk (Bosetti et al., 2006; Hedelin et al., 2006). Each those researches were carried out in EU populations in which flavonoid intake is low and may not attain chemopreventive degrees even inside the highest class of intake. These studies additionally differ within the tremendously large pattern length in comparison to that research that confirmed large inverse association with flavonoid intake. The chemopreventive effect of flavonoids in prostate most cancers has been in addition supported with the aid of some of massive prospective cohort studies that have verified an inverse association of cancer danger with flavonoid intake. In those studies, soy (RR0.3 for consumption of soy milk more than once in step with day), isoflavone (RR~0.5 in guys over 60 years of age) and green tea intake (RR0.52 for consumption of more than five cups according to day) was substantially correlated to

decrease prostate cancer risk (Kurahashi et al., 2008). However, one huge cohort examine performed in Japan did not exhibit an affiliation of inexperienced tea intake with prostate cancer (Kikuchi et al., 2006). This take a look at differed from the opposite massive jap cohort observe examining the effect of inexperienced tea intake (Kurahashi et al., 2008), by having a shorter observe-up time and a lower mean age of study contributors, each of which may additionally have prompted the likelihood of detecting a significant effect of green tea consumption. Two other cohort studies, both done on Finnish populations, did not report an association (Hirvonen et al., 2001; Knekt et al., 1997). In a observe-up to such a research but, a good sized inverse affiliation become proven for consumption of the flavonol myricetin and PCa. This highlights the impact of non- soy or inexperienced tea flavonoids in PCa chemoprevention (Knekt et al., 2002). Overall, the proof for a shielding impact of flavonoids in prostate cancer appears to be supported by way of the effects of maximum case-manage and cohort studies. Flavonoids have been the problem of controversy regarding their doubtlessly harmful phytoestrogenic effects in estrogen based malignancies such as breast and endometrial cancer. Notwithstanding this theoretical threat, the exceptional majority of epidemiologic studies have didn't display an improved breast cancer chance, and maximum in fact show an inverse affiliation with flavonoid consumption. These studies have examined breast and endometrial most cancers threat and the consumption of soy and green tea (both rich assets of flavonoids), or the unique flavonoids such as isoflavones observed in soy (genestein, daidzein), as well as flavonoids of other training inclusive of flavonols, flavones, anthocyanidins and catechins. Flavonoid intake was assessed in those studies via nutritional questionnaire or urine/serum dedication of flavonoid ranges. Fourteen out of nineteen case manipulate studies established notably lower flavonoid consumption in breast cancer sufferers compared to matched most cancers unfastened controls (Bosett et al., 2005; Zheng et al.,

1999).Four case-control studies confirmed no affiliation of flavonoid intake to cancer chance, while one look at established a moderate extended chance (RR 1.2) with daidzein intake in publish-menopausal women with breast cancer. In comparison to the protecting effect observed in case-manage studies, 5 out of six cohort studies failed to expose a significant association with flavonoid intake (Adebamowo et al., 2005; Travis et al., 2008). Single cohort study tested a defensive impact with excessive isoflavone intake (RR0.46). The absences of an association in different cohort studies can be explained by using variations in observe populations. The handiest effective have a look at was carried out in Japan in which isoflavone intake is significantly better than in the West (Yamamoto et al., 2003). The 5 poor studies have been conducted in EU or American populations wherein isoflavone intake is low and may not reach chemopreventive degrees even within the highest class of consumption. With reference to other estrogen-established cancers, three case control studies mentioned a sizeable inverse affiliation of soy or isoflavone consumption and endometrial cancer (Goodman et al., 1997; xu et al., 2004).

The useful outcomes of flavonoids in cancer are not confined to breast and prostate cancer. Results of potential cohort studies of flavonoid intake have normally been mixed in colon (Lin et al., 2006; Yang et al., 2007, gastric (Hoshiyama et al., 2002; Tsubono et al., 2001) and pancreatic cancer (Luo et al., 2007; Nothlings et al., 2007 2). One purpose for inconsistencies among research includes the range of nutritional screening tools for estimation of flavonoid consumption. The consequences are extra steady in ovarian cancer where a risk discount become mentioned for higher levels of isoflavone, black tea and flavonol (kaempferol and luteolin) intake (Larsson and Wolk, 2005). One large cohort take a look at of oral and pharyngeal cancer in Japan confirmed sizeable inverse affiliation with inexperienced tea consumption, but most effective in woman subjects (Ide

et al., 2007). Despite the fact that the anti-cancer results of flavonoids predominate, one most cancers type, bladder cancer, demonstrates an improved prevalence with better levels of flavonoid consumption. Three studies (one cohort and case-manipulate) have tested the intake of flavonols (quercetin, luteolin, kaempferol, myricetin), inexperienced tea consumption or soy isoflavones with respect to bladder most cancers chance (Sun et al., 2002). The most important of these research changed into a cohort have a look at performed in Singapore (n=63257), which established a substantially accelerated chance of bladder most cancers (RR= 2.32) with the best quartile of soy consumption (Sun et al., 2002). This seasoned-carcinogenic impact may be due in part to the urinary excretion of metabolized soy isoflavones, ensuing in excessive levels of changed seasoned-carcinogenic types of the flavonoids within the urine. The authors also proposed that other elements in soy can be accountable for the perceived pro-carcinogenic effect, because overall soy turned into extra quite correlated with bladder cancer risk than isoflavone concentration (Solar et al., 2002).

Some of research has examined the affiliation of non-flavonoid phytoestrogens which include lignans (e.g. Enterolactone) with prostate cancer risk and feature typically highlighted shielding effect of lignans. Due to the fact lignans are located in high stages in soy, tremendous epidemiologic institutions of the soy flavonoids (genistein and daidzein) with cancer may additionally indeed be a reflection of the confounding effect of lignans, or other as but unidentified phytochemicals (Hedelin et al., 2006).

1.1.7.3. Bioavailability and Metabolism of Flavonoids

The absorption, metabolism and excretion of flavonoids is a complicated process regarding diverse structural modifications to the ingested flavonoid in multiple tissues and cell compartments. Determining the bioavailability of flavonoids (the proportion of flavonoid located in blood or goal tissue after ingestion) is crucial to information the

outcomes of flavonoids as chemopreventive dealers. Even though flavonoids had been proven to undergo massive metabolism, this does not always equate to biological inactivation of the compound. In lots of cases in pharmacology, the metabolized product is often greater lively than the discern compound. A working example is morphine whose glucuronated metabolite is known to be a more potent opiate than the parent compound (Wittwer and Kern, 2006).

Flavonoids are predominantly absorbed within the small gut, with best small amounts absorbed thru the gastric mucosa (Piskula et al., 1999). The glycosylation kingdom of the flavonoid significantly influences the mechanism of flavonoid absorption. Maximum flavonoids in nature exist as glycosides. Early studies advised that flavonoid glycosides had been not absorbed intact in the human gut (due to their high hydrophilicity). These findings have greater recently been refuted (Griffiths and Barrow, 1972). Research has given that established that quercetin glycosides are not handiest absorbed, but that their absorption is absolutely stronger as compared to quercetin aglycone (Hollman et al., 1995). This absorption is believed to occur partly dueto the action of sodium structured glucose transporter (SGLTI) (Walgren et al., 2000). More usually, however, the first step in absorption of flavonoid-glycosides is typically hydrolysis of the sugar moiety in the intestine resulting in technology of the flavonoid aglycone (Spencer et al., 1999). This hydrolysis became to begin with assumed best to arise inside the colon through micro organism considering that human beings lack the vital enzymes to hydrolyze the β-glycoside linkages of flavonoid glycosides. But, currently is has become clean that a extensive- specificity β -glucosidase enzyme in enterocytes and lactase phloridzin hydrolase in the small intestine brush border can hydrolyze those β-glycoside linkages (Day et al., 2003). Hydrolysis of flavonoid- glycosides has additionally been proven to arise inside the oral cavity (Walle et al., 2005). The flavonoid aglycones generated by

means of hydrolysis of the sugar moiety are extra lipophilic, and for this reason is extra quite simply absorbed in the intestine by passive diffusion. Flavonoids entering the colon go through a comparable hydrolysis by means of bacterial glucosidases (Griffiths and barrow, 1972). Flavonoids that reach the colon undergo ring scission of the aromatic ring after sugar hydrolysis, resulting in simple phenolic compounds, which accounts for the low levels of flavonoid absorption from the colon (Rice et al., 2001).

The type of sugar moiety is an crucial determinant of absorption efficiency, with flavonoid-glucosides plenty extra conveniently absorbed than flavonoid-rutinosides (Hollman et al., 1999). With admire to the flavonoid aglycones, methoxylated flavonoids instead of hydroxylated flavonoids are tons more without problems absorbed thanks to their improved lipophicity, flavonoids lack an energetic transporter, and as such they are absorbed via passive diffusion, a extra green manner in hydrophobic flavonoids (Walle, 2007). Other elements affecting flavonoid absorption encompass the protein content of meals ingested with the flavonoid. For the reason that flavonoids bind to proteins, flavonoid absorption will be attenuated till the protein is digested (Prasain et al., 2007). The biotransformation of flavonoids continues in the enterocytes. The primary metabolic differences consist of conjugation of glucuronic acid (glucuronidation), methylation and sulphation (Spencer et al., 2004). These conjugations are basically phase ii detoxification reactions ensuing in improved molecular mass and progressed solubility of the compound which enhances excretion of the compound in bile and urine (Williamson et al., 2000). Hence the enterocyte is a crucial for flavonoid metabolism. Flavonoids aglycones that attain circulate are certain to albumin. Apparently, binding to albumin does not affect the antioxidant capability of flavonoids, an important point in terms of the likely biological impact of absorbed flavonoids (Janisch et al., 2004). Flavonoids entering the stream finally go through segment ii detoxification within the liver. Different differences inside

the liver consist of the formation of flavonoid-glutathione adducts also resulting in more suitable excretion of the flavonoid (Spencer et al., 2004). Excretion of flavonoids happens in urine as well as bile (Nielsen and Williamson, 2007). The kinetics of flavonoid absorption and metabolism in people has been studied for quercetin and other flavonoids. Hollman et al proven that height plasma ranges of quercetin had been reached in 2.9 hours in topics eating a meal of 333 grams fried onion (Hollman et al., 1996). The mean height plasma degree of quercetin was 196 ng/ml and the 1/2-existence of quercetin turned into 16.8 hours. This long half of-lifestyles show that quercetin might also acquire with persevered flavonoid administration. Importantly for functions of chemoprevention, research has additionally tested the buildup of flavonoids in numerous animal tissues (Chu et al., 2004). Quercetin, a flavonol, is believed to have specific absorption and kinetics to different flavonoid lessons. Anthocyanins, in evaluation, are absorbed poorly and swiftly excreted in urine. Citrus flavanones are properly absorbed but have shorter plasma half of lives. They also attain better maximum concentrations than flavonols (Manach and Donovan, 2004). A giant degree of variability is consequently predicted within the pharmacokinetic houses of various flavonoids. As with other ingested compounds, inter-person variability is any other critical thing accounting for the pharmacokinetic homes of flavonoids in human beings. Meals instruction has variable outcomes at the bioavailability of flavonoids within the diet. Peeling, as an instance, substantially reduces flavonoid content material for the reason that peel consists of a large proportion of flavonoids in end result and veggies (Gennaro et al., 2002). The impact of cooking on flavonoid content material has been examined for quercetin content in onions after cooking. While onions are boiled, flavonoids diffuse out to go into the broth, making the broth a rich supply of flavonoids. Frying onions for 40 mins did no longer adjust overall quercetin content. A growth in quercetin is mentioned on microwaving because of accelerated extractability

(Nemeth and Piskula, 2007). Consumption of flavonoids and protein collectively, while postulated to reduce the absorption of flavonoids, has no longer been proven to have an impact (Hollman et al., 2001). Accordingly, in fashionable, flavonoid availability from food appears to be enhanced through cooking.

1.1.7.4. Are flavonoids safe for human use?

The availability of a diverse range of flavonoids to most people as prescription-free dietary supplements propels the difficulty of flavonoid protection to the leading edge of public health concern. A large quantity of research has investigated the protection of flavonoids, especially of the isoflavone genistein, the green tea catechin egcg and the flavonol quercetin. The effect of these flavonoids has been investigated the usage of numerous genotoxicity assays in vitro and in vivo, and the protection of long-time period administration of those compounds in animals and humans has been assessed (Brown. 1980; Yamakoshi et al., 2002). Research examining the toxicity of EGCG proven no mutagenicity in a salmonella mutagenicity assay *in vitro*, however at doses of 210 μm had been clastogenic. Whilst administered to rats, EGCG become non-toxic at 50mg/kg as an intravenous bolus and became non-toxic to rats after 13 weeks administration at doses of 500mg/kg/day. An oral dose of two grams/kg become deadly in rats (Sbrucker et al., 2006). On the way to decide reproductive toxicity, mice were administered 14000ppm EGCG for the duration of gestation (Isbrucker et al., 2006). Even though there has been reduced domestic pup growth and pup loss, there was no proof of teratogenecity. 800mg/day of EGCG administered to people for four weeks had handiest minimal aspect effects along with mild gastrointestinal symptoms and muscle aches (Chow et al., 2006). There has been no hematologic or serum chemistry abnormalities. For this reason, egcg seems to be non-mutagenic besides at very high doses *in vitro*. EGCG ought to be avoided at some point of pregnancy. Genistein had no mutagenic effect in the Ames test,

and although clastogenic *in vitro*, there was no growth in micronuclei in wistar rats administered 2 grams/kg of genistein (Michael et al., 2006). Like EGCG, genistein administration in the course of gestation (at 1gram/kg) led to elevated pup mortality without being teratogenic (Mcclain et al., 2007). Research examining consequences of soy little one formulation have no longer validated any detrimental consequences in terms of growth, development or later replica (Merritt and Jenks, 2004). Genistein administered to humans at 600mg/day for several weeks did no longer result in micronucleus formation or rearrangement in MLL gene (Miltyk et al., 2003). Mcclain et al tested the impact of brief and long term administration of genistein via rats at a dose of 500 mg/kg/day (Michael et al., 2006). This dose became toxic to rats as evidenced via reduction in body weight and negative feeding. Long time management (52 weeks) became associated with improved liver gamma glutamyl transferase and minimal bile duct proliferation suggestive of liver toxicity. Consequences on hormone touchy tissues protected ovarian atrophy, hyperplasia of endometrial epithelium and vacuolation of epididymal epithelium suggestive of a estrogenic impact of excessive dose genistein on those tissues (Michael et al., 2006). In precis, quick term excessive dose (2 grams/kg) genistein isn't genotoxic *in vivo*, no matter being clastogenic *in vitro*. Toxic results of genistein consisting of estrogenic facet consequences are determined at doses of 500mg/kg. A safe dose in human beings is 600mg/day, even though the adverse effects at the fetus have to ward off genistein use all through pregnancy. Quercetin has tested wonderful for mutagenicity in maximum salmonella mutagenicity assays (Harwood et al., 2007). Quercetin additionally induces micronucleur formation and chromosomal aberrations *in vitro*. As for genistein and EGCG above, *in vivo* genotoxicity isn't always observed. The several *in vivo* research inspecting quercetin toxicity have these days been reviewed (Harwood et al., 2007). No poisonous consequences of quercetin at doses up to one gram/kg body weight/day were

proven. In mice eating 2000mg/kg/day quercetin for two years, an increased incidence of renal adenomas and adenocarcinomas was noted, although this observation has not been noted in other long term studies of similar dose quercetin ingestion.

In summary, the extent of toxicity from flavonoids is dependent on dose. Very high doses (>1gram per kg) are possible to have toxic side effects. While over-the-counter flavonoid supplements are suggested at doses of approximately 14mg per kg, side effects from flavonoid self-supplementation in humans are improbable. Although high doses of flavonoids were not teratogenic in most studies, fetal loss was increased and pup weight was reduced. For these reasons, excess flavonoid ingestion should be avoided during pregnancy.

1.1.8. The Mechanisms of Action of Flavonoids

1.1.8.1. Antioxidant Potential

Reactive oxygen species are enormously reactive molecules with each physiologic and pathologic role. Reactive oxygen species can occur in the form of molecules with exceptionally reactive unpaired electrons called free radicals (e.g. Superoxide, o •-), or as non-radicals which are fantastically liable to form free radicals (e.g. Hydrogen peroxide, h2o2). They can exist in the body because of planned synthesis (e.g. Production by macrophages for bacterial killing), or as a result of unintentional manufacturing by metabolic tactics such cellular respiration in mitochondria, or through exogenous insults consisting of smoking (Halliwell, 1994). Many reactive oxygen species aren't in themselves relatively reactive; but, in the presence of unfastened heavy metal ions inclusive of copper and iron they generate tremendously poisonous radicals which includes hydroxyl ions (OH•). ROS are quite negative as they are able to assault lipids in cell membranes, proteins, carbohydrates, and DNA. The ensuing oxidative damage may play a role in getting old and persistent and degenerative ailment along with cancer

(Halliwell2007). Approximately 1-3% of the oxygen we breathe in the long run goes into making reactive oxygen species, resulting in a big burden of pro-oxidant free radicals that needs to be effectively removed (Sohal and Weindruch, 1996). The human frame is predicated on both endogenous and exogenous (nutritional) anti-oxidant structures to buffer the effect of the reactive oxygen species continuously produced by using metabolic techniques. Endogenous antioxidant systems include enzymes along with superoxide dismutase, which converts O2-• into H2O2, and glutathione peroxidase and catalase, that serve to dispose of H2O2. Non-enzymatic endogenous protection mechanisms actually have a great anti-oxidant effect, along with buffering by using plasma urate and plasma protein thiols. Moreover, the sequestration of heavy metal ions in binding proteins such as transferrin (iron) reduces the risk of formation of toxic hydroxyl radicals (Halliwell, 1997). Despite those many tiers of protection in opposition to reactive oxygen species harm, endogenous antioxidant structures are incompletely effective in elimination of all enactive oxygen species, in particular with the introduced insult of numerous environmental reactive oxygen species from smoking, air pollution etc. Exogenous antioxidant supplementation, from dietary resources therefore has a vital function in the prevention of oxidative stress in human physiology (Scalbert et al., 2005). Antioxidant phytochemicals constitute some of the most critical exogenous protection antioxidants in mammalian physiology. Up until the mid-1990's the dietary phytochemicals maximum prominently studied for his or her antioxidant properties have been vitamin C, E and the carotenoids (Scalbert et al., 2005). Polyphenols, which constitute a main goup of plant chemicals, most effective received interest for antioxidant effects within the last decade. Flavonoids, the most important group of polyphenols discovered in nature, appear to be in particular mighty antioxidants in vitro. The flavonoids, but, aren't all similarly effective, with particular structural requirements vital for the finest antioxidant impact. The

presence of a 2, 3 double bond within the c-ring, a catechol structure within the b-ring, and hydroxylation at position 3 and 5 of the A ring appear to impart increased redox potential (Bors et al., 1990).

The redox potential of quercetin became similar to ascorbic acid and more than the redox capability of uric acid (Jovanovic and Simic, 2000). Direct scavenging of free radicals is one of the major mechanisms of antioxidant interest via flavonoids. The ensuing aroxyl radical (flavonoid-O•) is more stable than other reactive oxygen species and gains similarly stability on reacting with a second radical to form a solid quinone form (Pietta, 2000). Several different mechanisms of antioxidant activity of flavonoids have been proposed including scavenging of transition metal ions (Mira et al., 2002), and inhibition of enzymes answerable for antioxidant manufacturing. In phrases of the latter property, flavonoids were shown to inhibit several pro- oxidant enzymes such as xanthine oxidase, glutathione s-transferase (Van et al., 2002), nitric oxide synthase (Raso et al., 2001), and NADH oxidase (Morre et al., 2001) amongst others. The antioxidant results of flavonoids in vitro are nicely installed, and have been showed the use of numerous methodologies (Jovanovic et al., 1994). However, studies examining the outcomes of flavonoids in human beings have established conflicting effects. Lotito et al these days reviewed studies analyzing the protective outcomes of intake of flavonoid rich ingredients or extracts on oxidizability of LDL in human plasma (Lotito and Frei, 2006). Studies reviewed covered those analyzing green tea, black tea and pink wine consumption. Conflicting effects were proven for each of these flavonoid wealthy meals products, with each superb and poor consequence. Variations among studies could be accounted for through differences in the amount and frequency of flavonoid-rich meals intake. Others brush aside the effects of research of this layout because of the likelihood that flavonoids

could be removed from LDL during the lipoprotein isolation and wash procedures, therefore yielding inaccuracies in the results (Halliwell et al., 2005).

In spite of inconsistencies while analyzing LDL oxidizability, research inspecting total antioxidant potential of human plasma after intake of flavonoid rich diets have almost all mentioned considerable boom (Cao et al., 1997) measuring markers of oxidative damage of lipids (F2-isoprostane) and DNA (eight-hydroxy-2'-deoxyguanosine) constitute vary methodologies hired to decide the proper antioxidant impact of flavonoids *in vivo*. Research using those methodologies have typically didn't show a protecting antioxidant effect of flavonoid rich diets on lipid peroxidation or oxidative DNA harm (Halliwell et al., 2005). Therefore, while research consistently exhibit an growth of plasma antioxidant capability with intake of flavonoid wealthy foods, studies measuring markers of oxidative lipid and DNA damage fail to demonstrate a protective impact. Simply all the research on this area has centered at the antioxidant effect of flavonoid rich food or extracts, instead of natural flavonoids. Consequently, conclusions approximately the antioxidant effect of flavonoids can't be drawn from these studies, since meals merchandise consisting of the ones examined also are wealthy resources of non- flavonoid antioxidants. Discrepancies among the sturdy antioxidant capacity of flavonoids in vitro and their antioxidant efficacy *in vivo* can be because of the poor absorption and giant metabolization of flavonoids leading to bad bioavailability. The serum degrees of other nutritional antioxidants together with vitamins c and e are generally over a hundred instances greater than flavonoids even after the consumption of flavonoid wealthy meals (Lotito and Frei, 2006). Consequently, in ordinary human body structure, the actual impact of flavonoids on the entire antioxidant capability of human serum is likely to be minimal. consequently, regardless of flavonoids constituting the most important organization of antioxidants in

plants, a diet high in fruits and vegetables is more probable to give actual antioxidant supplementation in the form of non- flavonoid compounds.

1.1.8.2. Pro-Oxidant Effects

At the same time as flavonoids are exceptional recognized for their anti-oxidant homes, it has been shown that underneath certain conditions, flavonoids can be pro-oxidant. This property has been proposed to account for several biological results of flavonoids, consisting of apoptosis, which are induced inside the placing of oxidative pressure. These pro-oxidant consequences have additionally been observed for other phenolic antioxidants along with tocopherols, ascorbate, urate, curcumin and N-acetylcysteine. The balance between anti-oxidant and pro-oxidant consequences of these compounds is depending on several factors inside the cellular environment, in particular at the presence of transition steel ions.

The hydroxyl companies of flavonoids account for lots in their antioxidant impact. After scavenging reactive oxygen species, flavonoids are themselves oxidized, with a hydroxyl institution now containing a free radical referred to as a phenoxyl radical. A few flavonoids own a catechol form inside the B- ring. Oxidation of those flavonoids may result in a semi-quinone radical. The flavonoid semi- quinone can undergo in addition oxidation ensuing in flavonoid quinone. Consequently distinctive sorts of oxidation of flavonoids arise depending on their genuine structure. Similarly to scavenging of reactive oxygen species, flavonoids can also be oxidized in other methods. These encompass oxidation by means of cellular peroxidases, or by way of vehicle-oxidation inside the presence of oxygen- a method greatly extended within the presence of transition metal ions. The paradox of ‘antioxidant’ flavonoids is that in the process of scavenging reactive oxygen species, they become pro-oxidant radicals themselves, albeit less reactive than the scavenged species. The flavonoid radical’s never-the-less has undesirable properties. As

an instance, inside the presence of transition metals which includes Cu2+, flavonoids undergo a series of redox reactions culminating within the genesis of damaging hydroxyl radicals (Cao et al., 1997). Some other mechanism includes the flavonoid- quinones that are the products of oxidation in catechol containing flavonoids. Those are distinctly reactive to thiol companies, and result in the formation of flavonoid conjugates with thiol containing proteins which include glutathione. Interestingly, flavonoids that don't shape flavonoid-quinones have additionally been proven to shape thiol conjugates, highlighting the complexity of flavonoid chemistry that remains to be absolutely elucidated (Michels et al., 2004). The pro-oxidant consequences of flavonoids were shown to result in DNA harm and lipid peroxidation in vitro. Pro-oxidant radicals had been proven for numerous flavonoids which include myricetin (Bodnick et al., 1986), quercetin (Metodiewa et al., 1999), proanthocyanidins (Shao et al., 2003), inexperienced tea catechins (Azam et al., 2004), daidzein (Choi, 2006) and baicalin (Woo et al., 2005). The suitable situations promoting either the anti-oxidant or pro-oxidant effects of flavonoids *in vivo* remain to be addressed, and is a critical vicinity of future research. Most of the research of the oxidant homes of flavonoids had been executed in cell free or cell culture system. The dose of flavonoid utilized in this research is regularly over 10 fold better than is physiologically plausible by using diet or flavonoid supplements. Cell culture models additionally suffer a number of deficiencies now not least due to the artificially excessive oxidative stress situations in cellular subculture, and the presence of free metallic ions which can be regarded to set off seasoned-oxidant results in flavonoids (Halliwell, 2003). Various shielding functions of human physiology make it not likely that the pro- oxidant homes of flavonoids will have such dramatic consequences within the human. For example, flavonoid-quinone toxicity is quickly prevented *in vivo* by dithiols (Boots et al., 2005). Flavonoids also undergo significantly less auto-oxidation *in vivo* as a outcome of the

sequestration of free transition metal ions in carrier proteins such as transferrin. Therefore, the pro-oxidant effects of flavonoids are predictable to be considerably less obvious *in vivo*.

1.1.8.3. Hormonal Properties

Together with the antioxidant results of flavonoids, the hormonal, and particularly the estrogenic results of flavonoids had garnered the greatest attention in flavonoid studies during the last 50 years. The estrogenic activity of flavonoids first knew to mild in the 1950's while it became located that sheep grazing on purple clover pastures had decreased breeding rates (Bennets et al., 1946). Red clover become located to include numerous isoflavones and the estrogen-like properties of isoflavones had shown to account for fertility disturbances in animals feeding on pink clover. Because of their estrogen-like activity, isoflavones are also classed as phytoestrogens. The excessive ranges of isoflavones in crimson clover may also serve as a protection mechanism proscribing the populace of grazers, and thereby imparting a survival gain to the plant. The reproductive outcomes of isoflavones are of substantial challenge to human diet due to the fact meals rich in isoflavones, consisting of soy merchandise, are consumed in huge amounts in the human diet. These and other safety problems of flavonoids were mentioned. Isoflavones (genistein and formononetin) were shown to displace radiolabeled estradiol from the estrogen receptor (ER) (Martin et al., 1978). Studies have also confirmed the partial agonist properties of isoflavones at the estrogen receptor the usage of transfected e-detail reporter assays (Mayr et al., 1992). Flavonoids from the flavone, flavonols, flavonone and chalcone classes are considerably weaker phytoestrogens than the isoflavones as determined by way of competitive binding assays (Kuiper et al., 1998). Of the 2 estrogen receptor isoforms, genistein has a 7-fold more binding affinity to ERβ than ERα, even though binding affinity is 20 and 3.7 fold much less than 17-β estradiol

(E2) (Kuiper et al., 1997). 3-dimensional structure evaluation confirmed that genistein certain to the ligand-binding website of ERβ is similar to the herbal ligand E2 (Pike et al., 1999). Moreover, the recruitment of er co- activators become changed by using genistein differentially with the ERβ-genistein complicated binding ER co-activators to a far extra degree than ERα-genistein (Routledge et al., 2000).

The differential binding of phytoestrogens to ER isoforms is of significance, considering each of the ER isoforms has been shown to have awesome capabilities in proliferation. On the promoters of positive proliferation genes, ERα and ERβ have opposite moves, with ERα being seasoned-proliferative and ERβ anti-proliferative (Heldring et al 2007). No matter the vulnerable binding of ERα, genistein induced ERα activation at concentrations of 1μm in a luciferase-reporter assay (An j et al., 2001). Therefore, at the physiologically attainable dose of 1μm, genistein is an activator of both ER isoforms. Genistein and different flavonoids such as quercetin display a biphasic proliferation pattern *in vitro*, such that at doses <1μm these compounds cause an increase in proliferation in ER positive cell lines, and at >10μm they are anti-proliferative (Vander et al., 2005). The proliferative effect of flavonoids at low doses became proven to be depending on ERα for the reason that effect became abolished through the ERα antagonist hydroxytamoxifen. Apparently, genistein activated ER mediated expression greater in cells co- transfected with ERα and ERβ, than in cells transfected with ERα on my own, suggesting that ERβ contributes to the agonistic effects of genistein, which goes against the predominantly anti-proliferative effect typically attributed to ERβ (Pettersson et al., 2000). The anti-proliferative activity of flavonoids at better doses is seen in ER-terrible cell strains, and is therefore independent of each er isoforms (Maggiolini et al., 2001).

Taken together, the information advice that at low doses, flavonoids may be seasoned-proliferative in an ERα and in all likelihood ERβ based style, even as neither ERα nor ERβ are essential for the anti- proliferative activity of flavonoids at higher doses. The correct physiologic results of flavonoids mediated by their binding to ERα and ERβ are yet to be absolutely decided. That is an critical region of future research due to the fact phytoestrogenic flavonoids are fed on in massive quantities within the human diet. If the estrogenic effects of these compounds are predominantly pro-proliferative at low concentrations, flavonoids ought to doubtlessly pose a fitness risk in terms of selling hormone based cancers. Flavonoids can also exert anti-estrogenic results by using diverse enzymatic mechanisms. Blocking the synthesis of estrogens through inhibiting aromatase is a longtime strategy in the remedy of breast cancer. Flavonoids have been shown to bind the active site of aromatase, and inhibit its feature, with flavones and flavanones, instead of isoflavones having the finest impact (Hackett et al., 2005). Different enzymes of notice in estrogen metabolism include sulfatase and 17β- hydroxydteroid dehydrogenase, each of which bring about activation on estradiol precursors, and which can be inhibited by flavonoids (Basly and Lavier, 2005). The stages of intercourse hormone binding globulin (SHBG) through flavonoids are likewise of importance, as excess SHBG may bind estrogen reducing its impact (Pino et al., 2000). The estrogenic results of isoflavones in people is supported through research demonstrating altered menstrual cycle duration in women eating day by day soy protein, a product wealthy in isoflavones (Kurzer, 2000). In spite of the definite estrogenic properties of flavonoids, epidemiologic studies have for the maximum element validated a protecting impact for high flavonoid intake and hormone dependent cancer. The diploma of dietary flavonoid intake, estrogenic properties of flavonoids and hormone established cancers is highly complex. The connection can't be defined completely with the aid of the estrogenic residences of flavonoids. Different

mechanisms of action want to be taken into consideration in attempting to apprehend the real effect of flavonoid intake on hormonal cancer risk in humans.

The similarity in structure of flavonoids to all steroid hormones raises the possibility of ligand binding of flavonoids to other individuals of the nuclear steroid receptor family. Flavonoids had been shown to bind and prompt some of nuclear receptors along with androgen (Beck et al., 2003), progesterone (Beck et al., 2003), thyroid (Ricketts et al., 2005), and peroxisome proliferator-activated receptor pparγ (Ricketts et al., 2005). Flavonoids functionally spark off androgen receptor mediated transcription, resulting in multiplied prostate cancer antigen, a major downstream androgen receptor regulated gene. Apigenin, a flavone, became the most effective flavonoid in upregulating psa expression (Zand et al., 2000). Interestingly, in a associated look at other flavonoids had been proven to have exactly the alternative impact and inhibit prostate specific antigen developed. It turned into concluded that unlike the estrogenic results of flavonoids, the outcomes on prostate specific antigen production did now not comply with a structure-function realtionship (Rosenberg et al., 2002). Genistein and quercetin had shown to result in ar activation, however, it's far obvious from those studies that the impact of flavonoids, like that of dihydrotestosterone is biphasic, with activation of ar happening at low doses and ar inhibition at better flavonoid doses (Ren et al., 2000) pathway by genistein. This consists of up-regulation of vitamin D receptor gene expression and pastime (Gilad et al., 2006), and inhibition of enzymes (CYP24) that convert 1,25-vitamin D into much less active metabolites (Farhan et al., 2003). The vitamin D pathway is increasingly more implicated in chemoprevention of PSa, and up-law of this pathway is a doubtlessly useful synergistic property of flavonoids. Genistein has additionally been shown to have a stimulatory effect on insulin secretion *in vitro* (despite the fact that this impact is not seen *in vivo*) and an inhibitory effect on leptin secretion in rats administered genistein

(Szkudelska et al., 2007). Flavonoids as well inhibit corticosteroid secretion *in vitro* and *in vivo* (Szkudelska et al., 2007). In the end, the goitrogenic pastime of soy is properly documented, especially in the putting of iodine deficiency. This impact is assumed to be secondary to inhibition of thyroid peroxidase, a primary metabolizing enzyme in thyroxine biosynthesis (Doerge and Sheehan, 2002). Average, the interactions of flavonoids with steroid hormone pathways are incredibly complex. But, the impact of flavonoids on those pathways has been shown to in the end purpose alterations resulting in beneficial outcomes including the negative regulation of proliferative stimuli. As with a whole lot of flavonoid studies, the *in vivo* effects of flavonoids on hormonal signaling require further take a look at. That is highlighted in a current review by means of Hamilton-Reeves et al, in which most people of intervention studies reviewed did not discover a distinction in circulating intercourse steroid hormone stages (Hamilton-Reeves et al., 2007).

1.1.8.4. Cell Cycle Effects of Flavonoid

1.1.8.4.1. The cell cycle- background

The mammalian cell cycle is split right into a DNA synthesis (s section) and mitotic section (M-section) that are preceded by means of two gap stages, G1 and G2 respectively. The primary coordinators of the cell cycle are the cyclin structured kinases (CDKS). Cyclin structured kinases are activated through binding to precise cyclins, which leads to down-move changes that enable the cellular to progress from one segment to another. Cyclin structured kinases are expressed throughout the cell cycle, at the same time as expression of cyclins fluctuates with the one of a kind level of the cycle. The unique cyclin/cdk complexes of the G1 to s transition are cyclin D-cdk4/6, and cyclin E-cdk2. The primary goal of those kinase complexes is phosphorylation of the retinoblastoma protein (RB). Phosphorylation of RB reasons it to dissociate from the E2F transcription elements which could then sell G1 to S transition by the transcriptional up-

law of downstream genes. S-section progression calls for cyclin A-cdk2/cdk1 (cdc2) complex and G2 to M calls for cyclin b/cdk1 (cdc2) activation. Cyclin- cdk complexes are regulated through individuals of the cip/kip own family of pan-cdk inhibitory proteins (p21cip1, and p27kip1). Those proteins bind to cdk ensuing in inhibition of kinase interest. During the distinctive levels of the cell cycle, a number of fail-secure mechanisms exist called cell cycle checkpoints. These are signaling pathways important for the orderly and blunders unfastened progression of the cell cycle (Chang et al., 2004). Cell cycle arrest happens when checkpoints are activated through stresses such as genomic damage. Activation of checkpoints arrests the cell cycle thereby permitting time for DNA repair, or the activation of mobile death pathways in cases of irreparable insult. The activation of cellular cycle checkpoints results in cell cycle arrest through numerous pathways each submit-translational and transcriptional. Those pathways always lead to a modulation of cyclin-cdk complexes- the vital and very last determinants of cell cycle development. The DNA damage checkpoint is a classical example of the complex events that lead to checkpoint activation. ATM, a phosphatidylinositol 3-kinase like protein kinase, is an early sensor of DNA double strand breaks and a key player inside the DNA harm checkpoint (Khanna et al., 2001). ATM is mutated in the rare inherited disease, ataxia telangectasia. This disease is characterized via cerebellar degeneration, immunodeficiency, cancer predisposition and genomic instability (Shiloh and Kastan, 2001). ATM is activated by using a spread of agents, inclusive of ionizing radiation (IR) that set off DNA double strand breaks (Mcgowan and Russell, 2004). This triggers autophosphorylation of ATM on serine1981, and conversion of ATM from the inactive dimeric form to the active monomeric shape (Bakkenist and Kastan, 2003). ATM, phosphorylates a number of downstream proteins that result in cellular cycle checkpoint activation, and arrest at G1/S and G2/M stages of the cellular cycle. Among the

downstream phosphorylation objectives of ATM, are p53, mdm2, chk2, and h2ax (Maya et al., 2001). ATM phosphorylates p53 on serines 9, 15, 20 and forty six, ensuing in expanded stability and pastime of p53 (Saito et al., 2002). Phosphorylation of the p53 poor regulator mdm2 on serine 395 is likewise ATM, structured (Maya et al., 2001). This phosphorylation has been proven to minimize the moves of mdm2, thereby making sure a twin mechanism wherein p53 is activated and is chargeable for G1/S phase mobile cycle arrest. P53 has also been shown to steer the G2/M checkpoint through inhibition of cyclin B1/cdc2 pastime and through transcriptional upregulation of extra downstream target genes that prompt the G2/M checkpoint (Park et al., 2000). The G2/M checkpoint is regulated by the cyclin structured complex, cdc2-cyclin B1. Cdc2 is maintained in an inactive shape by using phosphorylation of residues threonine 14 and tyrosine 15 via wee1 and Myt1 kinases (Fattaey and Booher, 1997). Dephosphorylation of those residues by means of cdc25c turns on cdc2 and is a prerequisite for mitosis (Busino et al., 2004). Further activation of cdc2 occurs through phosphorylation of threonine 161 by way of cdk-activating kinase (cak). The checkpoint protein chk2 is a key participant on the G2/M checkpoint, and is activated with the aid of ir in an ATM-dependent way by phosphorylation at threonine sixty eight. Chk2 is liable for phosphorylating cdc25C at serine 216 inflicting inactivation of cdc25C by permitting 14-thre3-3σ binding, anchoring cdc25C inside the cytoplasm (Ahn et al., 2004). It is consequently clear that there is a close interplay between cell cycle proteins and upstream regulators in the shape of DNA harm sensors such as ATM.

1.1.8.4.2. Cell cycle alterations by flavonoids

Most flavonoids studied up to now were proven to goal the cellular cycle. Flavonoid treatment outcomes in an extensive variety of cell cycle changes along with inhibition of cyclin-established kinases and cyclins, or up-regulation of cdk-inhibitors of the cip/kip

circle of relatives. Alterations within the regulators of G1 to s transition along with RB and E2F proteins have also been validated. Flavonoids have additionally been shown to alter cell cycle checkpoint pathways, particularly the DNA harm response pathway. Numerous flavonoids were studied in prostate cancer *in vitro*. Apigenin, a flavone, has been studied in three prostate most cancers mobile strains –PC3, LNCaP and DU145 (Shukla and Gupta, 2007; Shukla and Gupta, 2006; Gupta et al., 2001). This flavone has been shown to induce G1 cell cycle arrest in all three cell traces. The molecular mechanisms of apigenin have been established and encompass down-law of cyclins D1, D2 and E, and inhibition of cdk2, four, 6. This became associated with up-law of p21, p27 and a reduction in phosphorylated Rb (Shukla and Gupta, 2007; Gupta et al., 2000). The green tea catechins have also been studied in those cell types and like apigenin, brought about a g1 arrest in all three cellular sorts (Gupta et al., 2001; Gupta et al., 2003). Similarly, the authors established an up-regulation of p16 and p18 (Gupta et al., 2003). In contrast, genistein, a soy isoflavone, prompted G2 arrest in PC3 and DU145 cells (Oki et al., 2004; Choi et al., 2000) and G1 arrest in LNCaP (Shen et al., 2000). The G2 arrest of genistein is defined by way of inhibition of cyclin B1, cdk1 and up-regulation of p21 and p27 (Choi et al., 2000). Silibinin, a flavono-lignan from milk thistle, prompted a G1 arrest in DU145 and LNCaP and a G1 and G2 arrest in PC3 (Zi and Agarwal, 1999; Deep et al., 2006). Different flavonoids studied in prostate cancer consist of quercetin (G2/M in PC3 and LNCaP) (Kobayashi et al., 2002), baicalin (G1 in lncap) (Ikezoe et al., 2001), acacetin (G1 in LNCaP and G1 and G2/M in DU 145) (Singh et al., 2005). The effect of flavonoids on cell cycle is cell- type structured. Apigenin, which arrested 3 PCa cell lines in G1, has been proven to arrest bladder cancer cells in G2. Hence, prediction of the kind of cell cycle arrest by using flavonoids is hard, and in all likelihood depends on numerous factors such as p53 repute of the cell lines being examined. Modulation of the ATM

dependent DNA harm checkpoint by way of flavonoids has been confirmed in several cancer types however has now not been tested in prostate cancer. Genistein turned into proven to arrest hepatoma cells in G2/m, an effect mediated through decrease in cdc2 kinase interest and activation of the ataxia telangectasia mutated (ATM) gene (Chang et al., 2004). Up-regulation of the ATM DNA damage reaction pathway by genistein represents a constraint to the development of genetic instability normally seen in pre-malignant cells. Silibinin has also been proven to spark off tumor suppressor p53 by way of the atm-chk2 pathway (Tyagi et al., 2006). Kaempferol, apigenin and luteolin have all been proven to prompt the ATM pathway in oral cancer cells *in vitro* (O'prey et al., 2003). Flavonoids are recognized to inhibit DNA topoisomerase ii that is chargeable for the clastogenic (DNA strand breaking) properties of flavonoids. The clastogenic properties of flavonoids occur at high micromolar concentrations and it is undetermined whether or not the pretty low doses of flavonoids in these research causes DNA strand breaks. It remains to be decided whether or not ATM pathway activation with the aid of flavonoids is genuinely a result of the DNA destructive outcomes of flavonoids, or whether or not the activation of ATM with the aid of flavonoids happens via a novel mechanism.

The activation of cellular cycle checkpoints has been proven to arise early inside the system of tumor genesis. This has been proposed to behave as a barrier against genetic instability and further malignant transformation (Bartkova et al., 2005). Flavonoids as a result have a direct effect on key cell cycle regulatory mechanisms that generally exist to suppress the proliferation of cells with tumorigenic potential. These properties of flavonoids may explain their chemopreventive effect highlighted in epidemiologic research.

1.1.8.4.3. Apoptotic Effects as a Consequence of Flavonoid Treatment

In addition to the cellular cycle and DNA damage pathways outlined above, flavonoids were shown to induce apoptosis in a ramification of cellular types. Apoptosis occurs through two properly- characterized pathways: the outside pathway that's initiated by using ligand binding to cell membrane loss of life receptors, and the intrinsic pathway induced by using modifications in internal cell signals. Both pathways ultimately result in activation of the caspase cascade. Caspases are cysteine structured proteases that initiate the sequence of events culminating inside the apoptotic phenotype. Within the intrinsic pathway, apoptogenic stimuli reason cytochrome c release from the mitochondria, an occasion inhibited through BCL-2 and promoted by BAX (Cory and Adams, 2002).the ratio of BCL-2 to BAX is an important element in apoptosis development. Once inside the cytoplasm, cytochrome c binds to Apaf-1 which recruits ATP and caspase nine to shape the apoptosome. Caspase nine recruits pro-caspase three to the apoptosome and activated caspase 3 mediates cell dying (Budihardjo et al., 1999). The apoptotic pathway is modulated with the aid of several different proteins along with the inhibitor of apoptosis own family (IAP). Flavonoids are being shown to persuade some factors of this pathway. Apigenin became shown to activate the mitochondrial apoptotic pathway as evidenced by way of lack of mitochondrial BCL-2 expression, mitochondrial permeability, cytochrome c launch, and the cleavage of caspase3 and nine (Gupta, Afaq and Mukhtar, 2002). Quercetin potentiated trail triggered apoptosis via the extrinsic pathway in DU145 and LNCaP prostate carcinoma cell lines, and brought on apoptosis in a p53 unbiased style in pc3 cells, related to an growth in BAX protein expression and a decrease in BCL-x(l)and BCL-2 protein (Kim and Kee, 2007; Vijayababu et al., 2006). EGCG however has been shown to cause apoptosis by way of a p53- structured mechanism. EGCG mediated apoptosis in pc3 cells turned into attenuated by inhibition of

p21 and BAX via sirna. Inhibition of transcription component nfκb by EGCG main to expanded apoptosis has additionally been confirmed (Hastak et al., 2003). Other flavonoids shown to set off apoptosis via comparable mechanisms in prostate carcinoma cells encompass genistein, isoliquiritigenin, silibinin, baicalin, among others (Agarwal et al., 2007; Jung et al., 2006). A unique association of flavonoids and apoptosis has been the association of degree of apoptosis brought on by means of flavonoids (EGCG) and their potential to inhibit fatty acid synthase (FAS) activity. Fatty acid synthase is a key lipogenic enzyme over-expressed in cancer cells (Brusselmans et al., 2005). In summary, flavonoids result in apoptosis in cancer cells by numerous mechanisms regulating each the intrinsic and extrinsic pathway

1.2. Hypothesis

The most common cancer in men is the malignancy of prostate which is the major public health problem worldwide. Moreover, it is also increasing regardless of intensive research and drug development. Therefore, reducing growth and triggering apoptosis in tumor cells without damaging the surrounding tissue may be an effective strategy for the anticancer drug development. However, the conventional chemotherapy for prostate cancer is not free from side effects. Hence, the evaluation of complementary and alternate therapies is necessary to resolve the serious side effects. Interestingly, plants-derived products are found to possess antitumor properties and are relatively nontoxic which can help in reducing the disease progression with minimal side effects. Further, to identify flavonoids and understanding the molecular mechanism responsible for the survival and proliferation of androgen-independent prostate cancer cells is critical for future targeted drug development. A previous case-control study of diet and prostate cancer in Japan revealed that consumption of fish, soybean products, bean curds, and fermented soybeans was associated with decreased risk. Hence, in the present study, we selected two potent

flavonoids eupalitin and cirsilineol and we hypothesized that these plant derived compounds could be an alternative medicine for the anticancer drug development.

1.3. Aim and objectives

In order to achieve the specific aims, we first analyzed the spectroscopy studies such as FTIR, ^{1}H NMR and ^{13}C NMR of eupalitin and cirsilineol and then examined the anti-proliferative activity against androgen-independent human prostate cancer cells (PC3) using different cytochemical and molecular parameters. In addition, we performed *in silico* study to confirm the binding affinity of molecules with receptors.

To end these following objectives set.

- To evaluate the anti-proliferative activity of flavonoids inducing ROS generation and apoptotic induction against androgen-independent human prostate PC3 cancer cells
- To evaluate the effect of flavonoids inducing apoptosis through cell cycle check points and pro-apoptotic pathway.
- To carry out the molecular mechanism of flavonoids underlying triggering of apoptosis through modulation of pro-apoptotic and anti-apoptotic markers in apoptotic pathway.
- To analysis the chemical structure of flavonoids by different spectral methods and its binding affinity with androgen receptors of prostate cancer by *In silico* study.

1.4. Significance of the proposed work

- There is a need to identify such compounds, which are effective and show mechanistic anti-proliferative activity against PC3 cell line which will provide future prospects in the development of anti-cancer candidates and therapy in traditional system of medicine.
- These flavonoids are herbal in nature, if used as a therapeutic agent then it will cause no/least side effects.

- The therapeutic efficacy of these promising natural compounds can be significantly noticed in cells proliferation, modulating ROS generation, apoptosis and cell cycle progression in PC3 cell lines.
- This study also helped in establishing *in vivo* test systems for evaluation of anti cancer activity of promising natural compound and our study is helpful in

1.5. Review of Literature

Diets rich in flavonoids have been associated with reduced incidence and mortality of prostate cancer (PCa). The lowest incidence of prostate cancer worldwide is seen in populations consuming the largest amount of flavonoids (Ganry, 2005). In East Asian countries (China and Japan) diets are up to 100 times more abundant in flavonoids than in the West, due in part to the consumption of soy and green tea (Morton et al., 1997; Adlercreutz et al., 1993). Correspondingly, the incidence of PCa in China and Japan is 60-80 folds lower than in North America (J. Ferlay et al., 2004). Studies on Japanese migrants to the United States have shown that migrants born in Japan and living in the United States have a higher incidence of PCa compared to men living in Japan(Lyn-Cook et al., 1999). The incidence rates for Japanese Americans born in the United States increases further, approaching that of American white men. Although these studies are not definitive, they emphasize the importance of environmental, lifestyle and dietary factors on PCa incidence. A number of case-control studies have correlated increased flavonoid intake with a reduced incidence of a number of malignancies including prostate cancer (Ganry, 2005; Neuhouser, 2004; Lee et al., 2003).

Flavonoids comprise over 4000 structurally related polyphenols (Bohm, 1998), which are ubiquitous in plants, and ingested to varying degrees in the diet. The estimated average daily intake of flavonoids is up to 1 gram (Kuhnau, 1976). This by far exceeds the intake of other antioxidants such as vitamin E, and highlights the potential importance of

flavonoids in the diet. Flavonoids have been shown to possess a wide range of biological activity, including antioxidant (greater than vitamin C) (Noroozi et al., 1998), anti-inflammatory, anti-thrombogenic, and anti-angiogenic activity (Valachovicova et al., 2004). The anti-cancer properties of flavonoids have been demonstrated in a variety of cell types *in vitro* and *in vivo* (Le Marchand, 2002). Despite the large number of flavonoids, studies have focused only on a select few. The flavonoids most intensely studied in PCa to date are the soy isoflavones (genestein/ daidzein) (Sarkar and Li, 2003; Castle and Thrasher, 2002), the green tea catechins (EGCG-epigallocatechin-3-gallate) (Park and Surh, 2004; Adhami et al., 2003) and the milk thistle flavonones (silibinin/ silymarin) (Agarwal, 2000). Little is known of the biological effect of most other flavonoids (Manthey and Guthrie, 2002).

In an attempt to identify novel flavonoids with growth arresting properties in PCa cells, we have screened a number of compounds from each of the major flavonoid sub-groups. We have examined their anti-proliferative effect on the prostate cancer cell lines PC3 (androgen independent). We have identified a number of novel flavonoids with anti-proliferative and cell cycle effects in human PCa cells *in vitro*. The compound identified with the greatest anti-proliferative effect was the natural flavonoid Eupalitin and Cirsilineol.

1.5.1. Plant profile of eupalitin

Eupalitin is an O-methylated flavonol. It can be obtained from powdered leaves of *Boerhaavia diffusa*. It belongs to *Nyctinaceae* Family. The Molecular weight of this compound is 330.29. The Molecular formula of eupalitin *is* C_{17} H_{14} $O_{7.}$ It is yellowish green powder and soluble in methanol and DMSO. It is insoluble in water. Eupalitin is one of the O-methylated flavonols that has been detected in *Ipomopsis aggregatag* (Smith et al., 1971). Study has reported that O-methylated flavonoids exhibited increased cancer chemopreventive properties than the corresponding hydroxylated derivatives of molecules (Bernini et al., 2011). At the cancer promotion stage, methylated flavones inhibited the proliferation of cancer cells, but not normal cells, with greater potency than the unmethylated flavones (Walle et al., 2007). It has been shown that methylated flavonoid considerably increased the metabolic stability with membrane transport in the intestine and liver and thus, improving the oral bioavailability (Walle, 2007). Hence, methoxyflavones have properties that may make them predominantly valuable as cancer chemopreventive agents. Other study has reported the potential immunomodulatory property of eupalitin (Pandey et al., 2005); however, none of the study has reported the antitumor activity of eupalitin inhuman prostate carcinoma and its underlying mechanism.

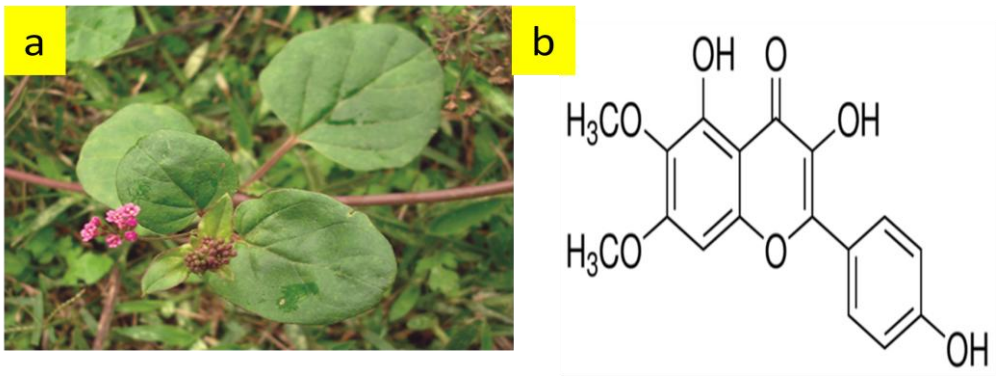

Figure 1.5 (a) *Boerhaavia diffusa* leaves- Source of eupalitin (b) structure of eupalitin

1.5.2. Plant profile of cirsilineol

Cirsilineol (4', 5-dihydroxy-3', 6, 7-thimethoxyflavone) is a bioactive compound isolated from dried whole plants of *Osmium sanctum*. It belongs to *Lamiaceae* Family. The Molecular weight is 344.32 and its molecular formula $C_{18}H_{16}O_{7.}$ It is pale yellow powder and soluble in alcohol and DMSO. It is insoluble in water. Cirsilineol (4^0,5-dihydroxy-3^0,6,7-thimethoxy-flavone) is isolated from the herb of *Artemisia vestita* Wall (Compositae), which has been widely used in traditional Tibetan and Chinese medicine for a variety of inflammatory diseases, such as rheumatoid arthritis, contact dermatitis and sepsis (Qiangba et al 2002; Wang et al 2005). Although this compound was reported more than 20 years ago (Maruhenda et al 1987), its biological activity has not been explored much except for antibacterial and antioxidant activity (Kelm et al 2000; Heo et al 2001; Isobe et al 2006). It has been reported that some flavonoids showed broad-spectrum anti-tumour effects (Kim et al 2004, 2005; Katayama et al 2007; Nam et al 2008). These findings drive us to explore a possible similar effect of cirsilineol. Cirsilineol considerably inhibited proliferation of Caov-3, Skov-3, PC3 and Hela cells in a dose-dependent manner. The cirsilineol also dose-dependently induced apoptosis in Caov-3 cells.(Xia Sheng et al 2008). In this study, we have first demonstrated that cirsilineol has anti-proliferative activity against various cancer cells by inducing apoptosis.

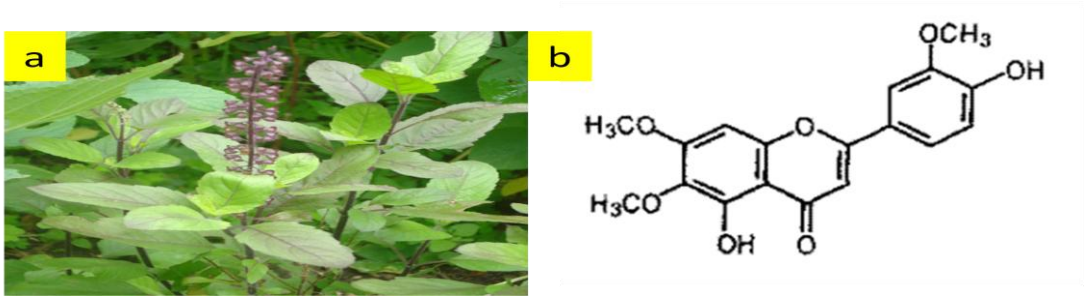

Figure 1.6(a) Osmium sanctum leaves - Source of cirsilineol (b) Structure of cirsilineol