1. General Introduction
And
Literature Review
In absence of an efficient primary health care system in many parts of the globe, traditional medicine occupies a central place in the provision of health care, especially among rural communities of developing countries. Traditional medicine is based on the strong bond between plants and humans. Plant species diversity and knowledge of their use as herbal medicines for up-keeping of human health is well substantiated in literature (Tabuti et al., 2003). In addition, herbal medicines are accessible and affordable (Steenkamp, 2003). From this root the natural products made a remarkable place in modern drug discovery and development program. In view of therapeutic potential of naturally occurring chemicals, flavonoids have gained a special identity as plants containing flavonoids have a long history of use in traditional medicines in many cultures. Flavonoids were discovered in 1938 when a Hungarian scientist Albert Szent-Gyorgyi used the term “vitamin P” to describe them. Flavonoids are a group of low molecular weight compound and based on the parent compound, flavone (Z-phenylchromone, or 2-phenylbenzopyrone) (Griffiths, 1982). They are widely distributed in plant kingdom like in fruits, vegetables, nuts, seeds, leaves, flowers and bark. Scultellaria root, cornus fruit, licorice, and green tea are examples of such flavonoid-containing foods and have been widely used in oriental medicine for centuries. The average daily consumption of flavonoids by a human has been estimated at about 1g; an amount that might be sufficient to give pharmacologically significant effect in vivo (Griffiths, 1982). Most flavonoids function in human body as antioxidants. They help to neutralize overly reactive oxygen-containing molecules and prevent other parts of cells. They may also exert several other mechanisms to protect the cell. One of them is the ability to increase levels of glutathione, a powerful antioxidant, as suggested by various studies (Valenzuela et al., 1989; Gandhi and Khanduja, 1993).

Flavonoids such as Apigenin, Genistein, Quercetin, Luteolin and Chrysin are present in our daily diets and explored by several investigators for their useful properties in prevention of various diseases. Synthetic flavone, flavopiridol, soy isoflavonoid (Genistein), the tea-epigallocatechin gallate or the common dietary flavonol (Quercetin) are emerging as prospective anticancer drugs and GTE-TP91 an extract of green tea already entered in clinical trials (Wang, 2000). Another flavonoid baicalein and its glycosides have been clinically used in China for many years (Wang, 2000). There is a great potential for discovery of new drugs and development of dietary
supplements from flavonoids. Antioxidant, estrogenic, anticarcinogenic and cardioprotective effects (Patel et al., 2007) of flavonoids also prompt a dramatic increase in flavonoids consumption as dietary supplements. In fact, in comparison to the wide spread use of flavonoids, very few systematic studies has so far been done on their toxic effects. A study on the toxicity of flavonoids in animals may increase our understanding to assess their safety. Therefore, detail study on three widely explored flavonoids (Apigenin, Genistein and Quercetin) having proven pharmacological effects were investigated in the present endeavor.

1.1. Apigenin

Apigenin belongs to the flavones subclass of flavonoids and chemically known as 4', 5, 7-trihydroxyflavone (Fig. 2 A). It is a low molecular weight flavonoid (Mw. 270.24) and its melting point is 347.5°C. Apigenin is a yellow crystalline solid in pure form, insoluble in water, moderately soluble in hot alcohol, and soluble in dilute KOH and DMSO. Apigenin is highly unstable in pure form and can be stored at -20°C (Budavari, 1997). Apigenin is widely distributed in fruits and vegetables such as parsley, onions, oranges, tea, chamomile, wheat sprouts and in some seasonings (Birt et al., 2001, Manach et al., 2004). Passion flower, which contains high levels of Apigenin, has been used effectively to treat asthma, intransigent insomnia, Parkinson's disease, neuralgia, and shingles. For centuries, passion flower tea and other preparations have been used for remediation of indigestion, gastritis, cutaneous inflammation and other dermatological diseases (Graf, 2000). Likewise, chamomile another rich source of Apigenin, is recognized as antiphlogistic, antispasmodic and antibacterial effects. It has been shown that Apigenin is having anti-mutagenic and anti-growth promoting properties that are due to inhibition of TPA-induced ornithine decarboxylase activity in mouse skin (Birt et al., 1986). These earlier studies with Apigenin generated further interest in development of Apigenin as a chemo-preventive and/or chemotherapeutic agent (Birt et al., 1986). Later Apigenin is shown to possess anti-spasmodic (Capasso et al., 1991), anti-diarrheal (Di et al., 1993), anti-inflammatory (Gerritsen et al., 1995), estrogenic (Breinholt et al., 2000a), antioxidant (Singh et al., 2004), anti-tumor (Cardenas et al., 2006) and anti-proliferative (Chiang et al., 2006) activities. A number of reports have been published describing the mechanisms of action of Apigenin which involve the generation of oxidative stress, mitochondrial dysfunction (Galati and O'Brien, 2004), transformation to reactive metabolites (Skibola and Smith, 2000; Silva
et al., 2000), DNA intercalation or the inhibition of DNA topoisomerase II (Yamashita et al., 1990; Ahmed et al., 1994a). Pro-oxidant behavior of Apigenin has been well studied (Chan et al., 1999, Galati et al., 2002, Morrissey et al., 2005; Miyoshi et al., 2007) and attributed to its structure (Galati et al., 2002). It is also argued that Apigenin as a polyphenol oxidizes by peroxidases to phenoxy! radicals that can co-oxidize b-nicotinamide adenine dinucleotide (NADH) or GSH and cause oxygen activation (O'Brien, 1988). Galati et al., 1999 reported the mechanism of Apigenin-mediated GSH oxidation, resulting in GSSG and superoxide radical anion. Miyoshi et al., 2007 demonstrated that Apigenin generates intracellular ROS and oxidizes protein carbonyl accumulation due to its 4'-hydroxyl group in human promyelotic leukemia cells; HL-60 cells. Wang et al., 1999 advocated that the position and number of hydroxyl groups of B phenyl ring of Apigenin strongly influence the conformation of target protein and modulates their inhibitory effect on protein kinases. In hepatocellular carcinoma cells (HepG2 cells), Apigenin at 20 μM concentration enhanced the generation of ROS in time-dependent fashion (Choi et al., 2007). In HL-60 cells, 50 μM of Apigenin for 1 h increased the intracellular ROS level up to 3.5-fold as compared to controls (Wang et al., 1999; Miyoshi et al., 2007). It is further demonstrated that Myeloperoxidase is constitutively expressed in HL-60 cells and converts Apigenin to phenoxy radical. Reactive oxygen species produced by Apigenin perturb mitochondrial function leading to mitochondrial permeability transition and cytochrome C release may induce apoptosis. Morrissey et al., 2005 advocated that the opening of mitochondrial permeability transition pore may be due to a loss of the anti-apoptotic mitochondrial Bcl-2 protein in prostate epithelial cells and demonstrated Apigenin cleaves the caspases 3, 7, 8, 9 and the inhibitor of apoptosis protein, cIAP-2.

Apigenin has been found to target the different intracellular signaling molecules involved in cell cycle and apoptosis. Oral intake of Apigenin resulted in dose-dependent increase in protein expression of WAF1/p21, KIP1/p27, INK4a/p16, and INK4c/p18, down-modulation of protein expression of cyclins D1, D2, and E, and cyclin-dependent kinases (cdk2, cdk4, and cdk6), decrease in retinoblastoma phosphorylation at serine 780 but increase in binding of cyclin D1 toward WAF1/p21 and KIP1/p27. Apigenin feeding resulted in stabilization of p53 by phosphorylation at serine 15 in 22Rv1 tumors which seem to exhibit p53-dependent growth inhibitory responses. In two independent studies Apigenin induced p53 expression that caused cell
cycle arrest and apoptosis in human cervical carcinoma cells (HeLa) and Hep G2 cells (Zheng et al., 2005; Chiang et al., 2006). Inhibition of CK2, a serine-threonine kinase, in Human colorectal carcinoma (HCT-116) and Human colon carcinoma (HT-29) cells by Apigenin affected a synergistic reduction in cell survival when used in conjunction with TNF-α, a multifunctional cytokine (Farah et al., 2003). Few recent studies have shown the cytotoxic (Matsuo et al., 2005, Zhang et al., 2008, Tsuji and Walle 2008) potential of Apigenin that may lead to apoptosis of human cancerous cells like prostate carcinoma cells (Gupta et al., 2001; Shukla and Gupta, 2006), cervical carcinoma cells (Zheng et al., 2005) and hepatoma cells (Chiang et al., 2006). Dimer of Apigenin, Amentoflavone, is also found to involve in production of reactive oxygen species that further suggest the Apigenin is a potential inducer of intracellular oxidative stress (Uddin et al., 2004).

Apigenin has been studied for its genotoxic effects. For example, Apigenin at a concentration 25 μg/ml (93 μM) is reported to enhance micronucleus formation alone or in the combination of single dose 2Gy137Cs gamma rays in human lymphocytes (Rithidech et al., 2005). Previous findings of our laboratory suggest that Apigenin increased the micronucleus frequency exponentially when the concentration of Apigenin was raised from 10- 100 μM in human lymphocytes, indicating potential clastogenic effects of Apigenin (Noel et al., 2006). Snyder and Gillies, 2003 also demonstrated the clastogenic effects of Apigenin at a dose of 100 μM in Chinese hamster V79 cells. The exact mechanism of Apigenin induced genotoxicity is not very clear and has to be investigated further beyond structure-activity relationship studies.

1.2. Genistein

Genistein (4', 5, 7-trihydroxyisoflavone) (Fig. 2 B) belongs to isoflavones, a subclass of flavonoid. It is yellow-white powder in physical appearance. Its molecular weight and melting point are 270.24 and 298°C respectively. It is soluble in DMSO and can be stored at -20°C for more than two years. Genistein is found in lupin, fava beans, soybeans, kudzu, and psoralea and also in medicinal plant like Flemingia vestita and coffee (Fukutake et al., 1996). Genistein is shown to improve bone health and immune function (Reinwald et al., 2010) and has antitumor (Cohen et al., 2000), estrogenic (Degen et al., 2002), antilipogenic (Borradaile et al., 2002), hypolipidemic (Banz et al., 2004), antioxidant (Rufer and Kulling, 2006) and anticarcinogenic (Sarkar et al., 2006) activities. Due to high production of soy based foodstuffs in Asia, Asian population is
in incessantly exposed to isoflavones. In United States, daily dietary intake of isoflavones averaged between 1.1 and 1.3 mg/day while it varies between 10 to 110 mg/day in China and Japan (Song et al., 2007). No such studies have been carried out in Indian population. However, Joshi et al., 2007 reported the plasma concentration of Genistein varied from 117-380 ng/ml at 4 to 8 h following single soy capsule intake containing 7.73 mg Genistein in Indian women. In 1999, the U.S. Food and Drug Administration recommended the daily ingestion of 25 g total soy protein (Safford et al., 2003), primarily due to reported beneficial effects on plasma lipid levels (e.g. lowered LDL, improved LDL/HDL ratios) thought to be crucial for prevention of coronary heart disease. The plasma or serum level of Genistein is reported to vary from 1-5 μM in humans after ingesting soy food (Maubach et al., 2003; Safford et al., 2003).

Genistein is a competitive inhibitor of tyrosine kinases and DNA synthesis-related enzyme topoisomerase-II (Strick et al., 2000). Due to this inhibiting function on these enzymes Genistein is implicated as a clastogen and a cytotoxicant (López-Lazaro et al., 2007). At concentration of 74-370 μM, Genistein is shown to inhibit topoisomerase II and relax pBR322 DNA (Okura et al., 1988). In primary hematopoietic progenitor cells, Genistein (50 μM) inhibited the activity of purified topoisomerase II (Strick et al., 2000). The threshold for topoisomerase II inhibition is found to be 96 μM in mouse lymphoma cells (Lynch et al., 2003). cDNA microarray analysis using LNCaP prostate tumour cells treated with Genistein (100 μM for 96 h) also showed a reduced expression of topoisomerase II mRNA upto 5 fold compared to untreated cells (Suzuki et al., 2002). Genistein induced Tk' mutations in cultured mouse lymphoma L5178Y cells at a concentration of 20 μM for 4 h. However, in presence of S9 fraction, doses ranging from 3 to 28 μM for 3 h did not induce Tk' mutations (Boos and Stopper, 2000 and Lutz et al., 2005). In 24 h exposure studies, Genistein is shown to be genotoxic at 18.5 μM, and cytotoxic at 24 μM in mouse lymphoma L5178Y cells in absence of S9 mix (McClain et al. 2006). Kulling et al. 1997 reported that mutation frequency is increased slightly in X-linked Hprt gene at 25 μM concentration of Genistein for 3 h in Chinese hamster V79 cells. Another study, using V79 cells, Snyder and Gillies, 2003 found that Genistein induced the formation of micronucleus in a dose dependent manner at concentration of 75-150 μM for 3 h with a response threshold of 10 μM. Following this study, Di Virgilio et al., 2004 found only CREST-negative micronuclei after the treatment of Genistein at 250 μM in V79 cells. Several other
reports demonstrated genotoxic ability of Genistein in different primary cultures of human and rodents (Record et al. 1995; Kulling et al., 1999; Pool-Zobel et al., 2000).

Genistein has potent and dose-dependent inhibitory effect on bovine brain-derived capillary endothelial cells and vascular endothelial cells derived from bovine adrenal cortex (ACE) or aorta (BAE) cell proliferation (Fotsis et al., 1993). Morris et al., 1998 found that Genistein induced apoptosis in AHH-1 tk+/− (mutant p53, p53+/−), and L3 (wild-type p53, p53+/+) cell lines. Genistein induced potent cytotoxicity and apoptosis in primary cortical neurons within 24 h of treatment at 50 µM concentration. It induced a DNA laddering pattern that is consistent with intra-nucleosomal cleavage resulting from caspase-activated endonucleases (Linford et al., 2001). Results of Choi and Lee 2004 are consistent with those of in vitro studies indicating that high concentrations of Genistein caused cytotoxicity and DNA ladder formation in primary cultures of cortical neurons. Genistein decreased the expression of caspase-3 (32 kDa) precursor and increased the levels of cleaved caspase-3 (18 kDa) in both rat brain tissue homogenates and in primary cultures of cortical neurons. Furthermore, expression of poly (ADP-ribose) polymerase (PARP) was also decreased in both experimental systems. These results suggest that chronic administration of Genistein at high doses may induce cytotoxicity and apoptosis in rat brain (Choi and Lee 2004).

In vivo toxicity studies using Genistein are limited. In mice, IP treatment of 50 mg/kg Genistein per 12 h for 3 days induced an increase in sister chromatid exchange in bone marrow cells (Giri et al., 1995). Additionally, in different rodent models studies have shown the multi-organ carcinogenic potential of Genistein (Rao et al., 1997; Newbold et al., 2001; Seike et al., 2002). In contrast to in vivo studies in male or female Moro albino mice, Wistar rats or RAIf rats did not demonstrate micronucleus induction at any Genistein concentration (up to 20 mg/kg/day for mice and 2000 mg/kg/day for rats) at either 24 or 48 h sampling times (McClain et al., 2006). The subcutaneous and oral lowest published toxic doses in female rat are 625 mg/kg and 1680 mg/kg respectively. These doses produced reproductive-specific developmental abnormalities-urogenital system and affect the newborn-growth statistics (e.g. % reduced weight gain) in female rats during 16-20 days after conception (JOENAK, 1955; PSEBAA, 1995). Yang et al., 2000 found that perinatal exposure of Genistein from 35 to 50 days of age was the root of mammary tumorigenesis in female Sprague-Dawley rats. Lowest published toxic dose in male mouse of 22-31 days pre-mating varied from 13200
mg/kg - 18600 mg/kg. This dose had an effect on Newborn-stillbirth and fertility-male fertility index (JOENAK, 1955). Genistein produced adverse effects at high dose of 500 mg/kg/day and were reversible in rats (McClain et al., 2006). McClain et al., 2006 observed that 50 mg/kg/day was relatively minor and, in view of functional (hormonally mediated) nature of effects, was not considered adverse effects. The increased incidence of minimal bile duct proliferation and slightly increased gamma-glutamyl transferase are indicative of a mild hepatic effect only at high dose of 500 mg/kg/day (McClain et al., 2006). In subchronic and chronic safety studies on dogs, Genistein was well tolerated and there was no evidence for systemic toxicity at doses up to 500 mg/kg/day administered orally for up to 52 weeks. However, some primary effects on the reproductive tract of both male and female dogs were observed that was anticipated and due to weak estrogenic activity of Genistein. The effects were reversible and functional in nature (McClain et al., 2005).

Most of studies on Genistein explored its estrogenic nature; its pro-oxidant nature is untouched. Like other polyphenols, Genistein has structural requirement to induce potent oxidative stress. At doses close to relevant levels, Genistein (10 μM, 24 h) induced a slight increase in oxidized DNA 8-OHdG levels in untreated MCF-7 breast tumor cells (Bianco et al., 2005). In another study, Genistein exhibited a variety of molecular events in 5-FU based combination therapy in HT-29 cells comprising the inhibition of cell growth, induction of apoptosis, ROS generation, inactivation of COX-2 expression and AMPK activation. In this study ROS was found as an upstream signal for AMPK activation by Genistein (Hwang et al., 2005). Salvi et al., 2002 have pointed out that ROS produced by Genistein could induce an acceleration of Ca^{2+} cycling followed by oxidation of pyridine nucleotides. Oxidized pyridine nucleotides are hydrolyzed by NAD-hydrolase and reaction products, ADP-ribose, opens a new specific pathway for Ca^{2+} efflux which enhances Ca^{2+} cycling. Yeh et al., 2007 found that ER stress was induced by Genistein involves the increase of calcium mobilization, cleavage of m-calpain, up-regulation of GRP78 and GADD153 expression, and activation of caspase-12. Genistein also induced the activation of executor caspase-3 and caspase-7. Moreover, the interaction with mitochondrial stress to downregulate Mcl-1 level and to generate truncated Bad may facilitate Genistein-mediated apoptosis in Hepatocellular carcinoma (Hep3B) cells (Yeh et al., 2007). Genistein was found to potentiate apoptosis induction by arsenic trioxide in human leukemia cells that was
mediated through ROS over-production and activation of oxidation-inducible protein kinases (p38-MAPK and AMPK) (Sanchez et al., 2008). More recent developmental toxicology studies in rat have demonstrated that dietary exposure to physiological concentrations of Genistein yields little or no toxicity (Flynn et al., 2000 a, b).

1.3. Quercetin

Quercetin (3, 3', 4', 5, 7-pentahydroxyflavone) belongs to the subclass flavonol of flavonoid. It is bright yellow crystalline solid having molecular weight 338.28 and melting point greater than 300°C. It is soluble in DMSO and ethanol. Quercetin is a common pigment in rinds and barks of wide variety of plants. It is one of the main flavonoids in diet, and is found in large amounts in apple skins, onions, tea, and red wine. Different amount of Quercetin is found in various foods viz. black and green tea (Camellia sinensis; 2000-2500 mg/kg), capers (1800 mg/kg), lovage (1700 mg/kg), apples (44 mg/kg), onion, especially red onion (1910 mg/kg), red grapes, citrus fruit, tomato, broccoli and other leafy green vegetables, and a number of berries, including raspberry, bog whortleberry (158 mg/kg), lingonberry (74-146 mg/kg), cranberry (83-121 mg/kg), chokeberry (89 mg/kg), sweet rowan (85 mg/kg), rowanberry (63 mg/kg), sea buckthorn berry (62 mg/kg), crowberry (53-56 mg/kg) and the fruit of the prickly pear cactus (Day and Williamson, 1999; Sampson et al., 2002; Hamly et al., 2006). It is also found in honey, herbs such as ginkgo and St. John's wort. Dietary supplements carry higher amount of Quercetin than would typically be found in food sources. Supplements are being marketed as capsules or tablets ranging in doses from 50-500 mg. Quercetin have different biological and pharmacological functions such as antioxidant, anti-carcinogenic, anti-inflammatory, cardioprotective and bacteriostatic (Gross et al., 1996; PDRNS, 2001; Erlund, 2004). Due to its therapeutic effects, Quercetin had been recommended by the United States government with 200-1200 mg daily intake in dietary supplements (PDRNS, 2001). Later a number of safety studies have been conducted by various International agencies (JECFA, 1977; PDRNS, 2001). However, in vivo toxicity data are meager (Harwood et al., 2007). Quercetin has been shown to induce gene mutations, chromosomal aberrations and micronucleus formation in different in vitro (Amacher et al., 1980a; Meltz and MacGregor, 1981; Carver et al., 1983; Van der Hoeven et al., 1984), and in vivo (Sahu et al., 1981; Nagao, 1981; Chaubey et al., 1982; MacGregor et al., 1983), however results were unequivocal. Different concentrations of Quercetin were tested through oral and IP routes in various
strains of mice (Sahu et al., 1981; Aeschbacher et al., 1982; Aravindakshan et al., 1985; Hang et al., 1985; Ishikawa et al., 1985; Hayashi et al., 1988; Caria et al., 1995; Ngomuo and Jones, 1996; da Silva et al., 2002) and rats (Aravindakshan et al., 1985; Taj and Nagarajan, 1996; Ngomuo and Jones, 1996; Cierniak et al., 2004; Tieppo et al., 2007; Utesch et al., 2008) and produced no genotoxic effects, however da Silva et al., 2002 and Sahu et al., 1981 reported genotoxic effects of Quercetin.

Bioavailability is one of the key factors in the potential toxicity provoked by Quercetin. Aglyconic form of Quercetin is rapidly absorbed and quickly bound in liver as glucuronides and/or sulfate conjugates that are excreted as such in urine, bile and feces (Kuhnau, 1976). When urine, feces and plasma samples were tested for mutagenicity, fecal extract showed highest mutagenic activity in Ames assay. S9 fraction, contains cytosolic and microsomal fraction with different enzymatic activities, has a major role in producing active metabolites of Quercetin following the enhancement of mutagenicity (Sugimura et al., 1977; Hardigree and Epler, 1978; MacGregor and Jurd, 1978). The revertants of *Salmonella typhimurium* strain TA100 were increased with S9 activation (Nagao et al., 1981). It has been found that Quercetin has structural requirements for genotoxic activity viz. hydroxyl groups (Fig. 2 C) at positions 3' and 4'. In Ames assay, metabolic activation was not required for mutagenic potential of Quercetin however it was enhanced in the presence of S9 mix (MacGregor and Jurd, 1978; Vrijsen et al., 1990; Gaspar et al., 1994). Different experiments were performed to evaluate the toxic potential of Quercetin in vivo models (Maruta et al., 1979; da Silva et al., 2002; Blaszczyk et al., 2007). Maruta and Nakayasu developed azaguanine (Maruta et al., 1979) and diphtheria toxin (Nakayasu et al., 1982) resistance markers respectively in different cell lines for an easy assessment of mutagenicity produced by Quercetin. Besides being a potent mutagen Quercetin is also reported as clastogen. It was found to induce aberrated chromosomes and sister chromatid exchange in vitro and in vivo systems (Sahu et al., 1981; Carver et al., 1983; Popp and Schimmer, 1991). Intercalative behavior of Quercetin was identified through spectrophotometric changes that suggested the interaction of Quercetin with DNA. As a result, Quercetin induced single strand breaks in calf thymus DNA (Ahmad et al., 1992), pancreatic islets (Omamto et al., 1981), mouse lymphoma L5178Y TK-/- mutation assay system (Amacher et al., 1980b; Meltz et al., 1981; van der Hoeven et
al., 1984), human lymphocytes, and in Hela cells (Duthie et al., 1997). Quercetin was also found to interact with plasmid DNA and capable to open it (Rahman et al., 1992).

DNA damaging frequency of Quercetin increased in the presence of transition metals (Ahmad et al., 1992; Sahu and Washington, 1992; Formica and Regelson, 1995). Strand scission in DNA by Quercetin-Cu (II) complex produced a uniform cutting pattern of inter-nucleotide bonds. This led to the observation that Quercetin-Cu (II) cleavage reaction has potential of being used as preferred DNA foot printing reagent (Ahmed et al., 1994b). Response of Quercetin in different short term assays appeared to suggest that Quercetin exerts DNA-damage via more than one mechanisms (Rueff et al., 1986): (1) reacting directly with the DNA (Rueff et al., 1992), (2) producing highly genotoxic metabolites upon metabolization by cytochrome P450 and cytosolic enzymes (Vrijisen et al., 1990; Rueff et al., 1992) and (3) producing oxygen free radicals (Rueff et al., 1992; Gaspar et al., 1994). Quercetin induced DNA cleavage with subsequent DNA ladder formation in HL60 cells. The same but less potential effect was found in the case of HP100 cells, an H2O2-resistant clone of HL-60 cells. Quercetin increased the formation of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), an indicator of oxidative DNA damage, in HL-60 cells but not in HP 100 cells. These findings suggested that Quercetin induces H2O2-mediated DNA damage, results in mutations or induction of mutation (Yamashita et al., 2000). DNA damage induced by Quercetin activates specifically error prone repair pathway components in prokaryotes. The different level of induction of SOS genes including sfiA, umuC and recA genes by Quercetin and its metabolites can apparently be explained by different affinities of binding of LexA protein to regulatory regions of different SOS genes (Walker et al., 1984; Llagostera et al., 1987). Apart from having DNA damaging potential, Quercetin inhibits the synthesis of DNA, RNA, as well as protein in Ehrich ascites tumor cells (Graziani and Chayoth, 1979) resulting in inhibition of growth (Yoshida et al., 1990). Potential to induce carcinogenesis is another characteristic feature of Quercetin. Different mouse and rat strains were used to examine the carcinogenic behavior of Quercetin (Pamukcu et al., 1980; Saito et al., 1980; Hosaka and Hirono, 1981; Hirono et al., 1981, Morino et al., 1982; Ertürk et al., 1983; Takanashi et al., 1983). A considerable amount of Quercetin is present in bracken fern that is consumed by cattle of Europe and initiates cancer in them (Sugimura et al., 1977). Treatment of primary bovine cells in vitro with a single dose leads to full oncogenic transformation of cells
transfected with Bovine papilloma virus type-4 and ras gene (Pennie and Campo, 1992). Quercetin induced morphological transformation of cryopreserved hamster embryo cells at concentration of 5 μg/ml and 10 μg/ml (Umebayashi et al. 1977). Moreover, Quercetin was found to be an intestinal and bladder carcinogen in Norwegian rats during administration for 1 year at 0.1% in the diet (Pamukcu et al., 1980). Quercetin alone as well as in combination with other flavonoids induced carcinogenicity (Morino et al., 1982).

Several researchers supported the fact that Quercetin possesses all structural elemental characteristic of an anti-oxidant (Metodiewa et al., 2005) but few were assuming that Quercetin may act as a pro-oxidant molecule (Metodiewa et al., 1999; Kaldas et al., 2003). It is believed that the pro-oxidant action may be responsible for in vitro mutagenic activity of Quercetin. Sahu and Gray, 1997 explained the Quercetin induced genotoxicity was the result of reactive oxygen species (ROS) production by redox cycling. Quercetin gives rise to the superoxide anion by auto-oxidation, which in turn may lead to the formation of H₂O₂ (Gaspar et al., 1993) and subsequently to DNA damage (Anderson et al., 1994). In in vitro system, Quercetin was shown to form free radical ortho-semiquinone intermediates, which may subsequently be converted to the parent compound or alternatively to an ortho-quinone, accompanied by the production of reactive oxygen species such as superoxide and hydrogen peroxide (H₂O₂) (Metodiewa et al., 1999; Boots et al., 2003). Oxidation of Quercetin by H₂O₂ in presence of horseradish peroxidase or tyrosinase to the reactive intermediates, orthoquinone/ quinone methides, was demonstrated in vitro (Boersma et al., 2000; Awada et al., 2002; Kaldas et al., 2005). Additionally the pro-oxidant behaviour of Quercetin is responsible for its binding to cellular DNA and protein in vitro following oxidation presumably by H₂O₂/peroxidase (Walle et al., 2003). For example 25 μM Quercetin was found to bind efficiently with human serum albumin (13.8 μM); however, the addition of glutathione (GSH) at normally occurring cellular concentrations (5 mM) was observed to completely suppress the binding (Kaldas et al., 2005). Aglyconic form of Quercetin was found to bind with DNA in cultured human cell lines (i.e. intestinal Caco-2 and hepatic HepG2 cells) (Walle et al., 2003; van der Woude et al., 2006) however, this binding capacity is decreased by methylation of Quercetin at 3' and 4' hydroxyl moiety up to 50% (van der Woude et al., 2006). Quercetin induced H₂O₂ and superoxide anion
in human lung embryonic fibroblast and human umbilical vein endothelial cultured cell depending on dose and free radical source (Matsuo et al., 2005). In human red blood cells, Quercetin was shown to oxidize some erythrocyte oxyhemoglobin, producing methemoglobin, with an increase in activity observed in the presence of extracellular horseradish peroxidase and H₂O₂, but did not markedly increase the incidence of hemolysis (Galati et al., 2002). Evaluated in vitro in rat hepatocytes with added H₂O₂ and peroxidase, Quercetin was demonstrated to rapidly co-oxidize ascorbate, which was likely mediated by the semiquinone radical; however, in contrast to its phenol B-ring-containing counterpart, Kaempferol, Quercetin did not co-oxidize reduced b-nicotinamide adenine dinucleotide (NADH) or GSH (Galati et al., 2002). The Quercetin-induced depletion of GSH levels that accompanied the oxidative reaction in isolated hepatocytes was determined to be due to GSH conjugation of quinone methide reactive products, rather than GSH oxidation. Thus, it does not appear that the Quercetin oxidative products oxidize GSH to form reactive thyl radicals (GS). Conjugation of Quercetin intermediates with GSH results the formation of mono-GSH and bis-GSH conjugates had been confirmed in an earlier study (Galati et al., 2001). In comparison to control group, a significant increase in the levels of hepatic and pulmonary glutathione-S-transferase (GST) and GSH levels were noted in an 8-week mouse study in which the test animals received up to 2.6 mg/kg body weight/day Quercetin via drinking water (Gandhi and Khanduja, 1993). In contrast, Duarte et al., 2001 reported a decrease in levels of liver GSH in Quercetin-treated (10 mg Quercetin/kg body weight/day by gavage) normotensive Wistar Kyoto rats compared to untreated controls, which was not accompanied by any changes in glutathione peroxidase (GPX) activity or glutathione reductase (GR) levels. Levels of liver and plasma malondialdehyde (MDA), a lipid peroxidation product, in Quercetin-treated rats were comparable to controls or slightly reduced (not statistically significant), respectively. In another study, increases in GST and GR activities were observed in segments of the colon mucosa of Sprague-Dawley rats maintained on Quercetin-supplemented diets (up to 1% of the diet or ~500 mg/kg body weight/day); however, no change was reported in liver enzyme activities (Fischer et al., 2002). Choi et al. (2003, 2005) assessed potential Quercetin related pro-oxidant activity in male Sprague-Dawley rats following 4-6 weeks oral treatments with up to 20 mg Quercetin/rat/day (approximately 78 mg/kg body weight/day). Quercetin was found to decrease hepatic levels of GSH significantly,
while concomitantly increasing, albeit only slightly and not at levels of statistical significance, the activity of GPX and decreasing GR activity compared to controls. Rangan et al., 2002 observed elevated levels of renal H2O2 production in male Wistar rats fed Quercetin in the diet for a period of 21 days at the dose level of 285 or 1133 mg/kg body weight/day. In another study in which Quercetin was administered to rats for a period of 5 weeks at the dose level of 10 mg/kg body weight/daily by gavage, plasma levels of thiobarbituric acid (TBARS) and nitrites/nitrites (NOx), markers of oxidative liver stress, were comparable to controls (Gale, et al., 2005). With the exception of a slight reduction in liver C2 levels in Quercetin-treated rats, no significant changes were observed in liver C2, GPX, or GR activity between test and control rats. Moreover, gavage administration of Quercetin at a dose level of 135 mg/kg body weight/day for 3 consecutive days did not significantly enhance the activity of oxidative scavengers, superoxide dismutase and catalase (Cierniak et al., 2004). Furthermore, the pro-oxidant behaviour of Quercetin provides clue of apparent in vitro mutagenic effect. Interestingly, available literature of in vivo protective mechanism efficiently limits any potential for adverse effects related to Quercetin pro-oxidant activity. Few additional studies are needed to further support the absence of dietary Quercetin-related carcinogenicity in vivo (Okamoto, 2005).

To understand the effects of these three flavonoids in liver and kidney, present studies were planned using Swiss mice. Liver and kidneys were considered as they receive 28% and 23% of total cardiac output respectively, greater than other organs and are the primary organs exposed to chemicals flowing in blood. Liver is responsible for metabolizing most compounds and can therefore be used to predict how drugs are metabolized, how they might interact with each other in body, and to what extent they or their metabolites may be toxic to liver. Even though the liver detoxifies various harmful substances to harmless by-products, certain bio-transformations often generate potentially toxic molecules different from their parent compounds. For targeting liver directly, flavonoids were administered through intraperitoneal route. Moreover, unique relationship of liver with gastrointestinal tract results in exceptionally high concentration of chemicals in the liver tissue. This may be particularly significant for xenobiotics whose site of action is liver. Similar effects are observed with certain therapeutics whose bio-transformation in liver often results in production of toxic by-products. The unwanted by-products often attain considerable concentration in hepatic
tissue, consequently resulting in target organ toxicity with a number of debilitating
effects and breakdown of general body homeostasis. Kidneys take part in general
homeostasis of body and get continuous blood flow. Drugs are regarded as a common
source of acute kidney injury. Drugs may damage the blood vessels, glomeruli and
different parts of nephron and interfere the renal functions. In addition, they may
directly or indirectly alter renal blood flow and its intrarenal distribution. In addition
this study also examined effects of flavonoids on liver and kidney at gene expression
level and explores to establish the mechanism of toxicity at higher doses.

Figure 2: Structures of selected flavonoids; A. Apigenin, B. Genistein, C. Quercetin