Chapter- 5
Discussion
DISCUSSION

In the present study, the chromosomal aberration, micronucleus assay and the comet assay were cooperatively used to study the genotoxic effect of iron sulfate and modulatory effects of pre, simultaneous and post treatment of TQ and QCT against iron sulfate induced genotoxicity at a chromosomal level, micronuclei level as well as a DNA level for 24 h. It has been reported that genotoxic chemicals in general produce the highest frequency of aberrations in rodents 24 h after single exposure, which roughly coincides with the normal length (22–24 h) of the cell cycle (Adler, 1984; Hayashi, 1984). Three genotoxicity evaluation methods cover different aspects of genotoxic events. Bone marrow chromosomal aberration is one of the most effective and sensitive short-term in vivo bioassay that utilizes cytological damage as an end-point in detecting and screening chemical agents that induce clastogenicity (Sanchez et al., 1988). Chromosome analysis of bone marrow cells in vivo is a standard method for testing for the potential mutagenic effects (Celik et al., 2003). The micronucleus assay detects chromosomal damage that persists for at least one mitotic cycle, while the comet assay identifies repairable DNA damage or alkali-labile sites (Zhong et al., 2001). Some of the DNA lesions, such as single and double strand breaks, and adduct formation, led to permanent fetal damage, and the micronucleus test is an excellent means of evaluating any permanent damage in genetic material. On the other hand, the comet assay is a very sensitive method for measuring DNA strand breaks at a single cell level and thus is sensitive to acute DNA lesions. Moreover, it was recently shown that the comet assay detected nearly 90% of carcinogens that were negative or equivocal in the micronucleus assay. Therefore, a combination of the chromosomal aberration, micronucleus assay and the comet assay is recommended for the broad assessment of in vivo genotoxic potential (Pfuhler et al., 2007; Kirkland and Speit, 2008). The National Toxicological Program (NTP) is presently using this combined protocol as part of its efforts to evaluate the genotoxicity of substances of public health concern.

In our experiments of first part we used iron sulfate alone since we aimed to determine the concentration at which iron exerts district genotoxic effects. When we evaluated the number of chromosomal aberrations in bone marrow using bone marrow chromosomal aberration assay, number of MNPCes (as genetic marker) in PCEs, in bone marrow
using the micronucleus assay, and the extent of DNA damage in terms of tail moment and olive tail moment in whole blood cells by the Single cell gel electrophoresis (SCGE/Comet assay), the number of chromosomal aberrations, the numbers of micronucleated polychromatic erythrocytes and the extent of DNA damage increased with increase in the concentration of iron sulfate.

Metal ions can generate DNA damage directly or induce the formation of reactive oxygen species (ROS), leading to DNA damage indirectly probably via Fenton-like reactions (Linder, 2001; De Freitas and Meneghini, 2001). Recently, we observed clastogenicity after acute exposure (24, 48 and 72 h) of human lymphocyte culture to iron sulfate in vitro as evaluated by the chromatid and chromosomal type aberrations (Parveen and Shadab, 2011). In addition, Pagano et al. (1996) demonstrated a high mutagenic and teratogenic potential of bauxite factory samples, where high levels of metals are found, including Fe. Low concentrations of Fe are not able to induce genotoxic effects, since this metal is a common chemical element of cells, and is essential for organisms as a co-factor in oxygen transport (Ganong, 1993; Nelson and Cox, 2002). However, at high concentrations, this metal presents a significant risk for the development of population disorders. Neoplasias and cardiac, pulmonary, hepatic, gastrointestinal and renal alterations are related to Fe exposure. This metal is also toxic to neural tissue and it is related to an increased risk for the development of neurodegenerative disorders (Chau et al., 1993; Lima, 2001).

In our study, as expected, there was a significant difference in the number of chromosomal aberrations, number of MNPCEs and DNA damage produced between the lowest (50 mgFe/kg) and highest concentrations (200 mgFe/kg) of iron sulfate. High levels of chromosomal and chromatid aberrations were found in human lymphocytes and TK6 lymphoblast cells exposed to high-energy iron ions (56Fe) (Evans et al., 2001; Durante et al., 2002; Evans et al., 2003). Glei et al. (2002) detected a significant DNA damage, determined by micro gel electrophoresis, in differentiated human colon tumor cells (HT29 clone 19A) incubated with ferric-nitrilotriacetate (Fe-NTA). Mutagenic activity was also found in elemental and salt forms of Fe, evaluated with the tests for mutagenicity in Salmonella typhimurium and L5178Y mouse lymphoma cells (Dunkel et al., 1999). It has also been reported that iron compounds are mutagenic in cultured mammalian cells, as detected by Syrian hamster embryo cell transformation/viral
enhancement assay (Heidelberger et al., 1983), sister chromatid exchange (SCE) in hamster cells (Tucker et al., 1993) and base tautomerization in rat hepatocyte cultures (Abalea et al., 1999). The MNPCES in young erythrocytes arise primarily from chromosome fragments that are not incorporated into the daughter nuclei at the time of cell division in the erythropoietic blast cells and changes in the incidence of MNPCES are considered to reflect chromosomal damage (Salamone and Heddle, 1983). The present study also reveals that high dose of iron increases the number of MNPCES in the polychromatic erythrocytes. In support of this in recent study the results show significant genotoxicity for iron at the high doses tested (Pra et al., 2008). Our comet assay results confirm that there is a significant increase in the extent of DNA damage in terms of tail moment and olive tail moment by the administration of iron sulfate in dose dependent manner. Many ideas about the physiology of metals have been questioned and there is growing evidence that copper and iron might be associated to DNA through in situ reactions, leading to genome damage (Tkeshelashvili et al., 1991; Meneghini, 1997).

Present study indicated that high dose of iron increases the number of chromosomal aberrations, MNPCES, and the extent of DNA damage in dose dependent manner. Oxidative damage is among the most potent and omnipresent threat faced by any living organism. Intracellular accumulation of reactive oxygen species can arise from toxic insults and can perturb the cell’s natural antioxidant defense system resulting in damage to all major classes of biological macromolecules. Therefore, arising attention is given to the study of natural products, which may counteract the detrimental effects of oxidative stress and prevent multiple human diseases. In this line, different types of fruits and vegetables have been re- evaluated and recognized as valuable sources of nutraceuticals. Generally, antioxidant and antimutagenic effects of medicinal plant extracts or their constituents are evaluated in vitro applying bioassays such as Salmonella typhimurium (Ferrer et al., 2001) or bone marrow cells, spleenocytes and human lymphocytes (Aboul-Ela, 2002). TQ is the bioactive constituent of the volatile oil of black seed (El-Dakhakhany, 1963). It exerts antioxidant effects and inhibits inflammation in animal models and cell culture systems. It is widely accepted that antioxidants, either endogenous or from the diet, play a key role in preserving health. They are able to quench radical species generated in situations of oxidative stress, either triggered by pathologies or xenobiotics, and they protect the integrity of DNA from genotoxicants. To
examine this issue, our present work was initiated to determine whether TQ and QCT protect the cells against the genotoxicity of iron. Different doses of TQ along with iron were found very effective in reducing the chromosomal aberrations, number of MNPCEs and DNA damage. The protective effects of TQ are likely due to its antioxidant properties (Mansour et. al., 2002). Iron excess is responsible to generate oxidative stress, and increases the steady state dose of reactive oxygen and nitrogen species. The toxicity of superoxide anion \((O_2^-)\) and hydrogen peroxide \((H_2O_2)\) arises from their iron dependent conversion into the extremely reactive hydroxyl radical \((OH^-)\) (Haber–Weiss reaction) that causes severe damage to membranes, proteins, and DNA (Halliwell and Gutteridge, 1984). Consequences include enhancement of radio sensitivity, mutation, lipid peroxidation, polysaccharide depolymerization, enzyme inactivation, degenerative aging and cell death (Weinberg and Miklossy, 2008). The present investigation demonstrated ameliorative effects of pre, simultaneous and post treatment of TQ against genotoxicity induced by iron sulfate. Accidental iron overdose is one of the most common causes of poisoning deaths in children under 6 years of age in the United State. Poisoning symptoms occur with doses between 20 and 60 mg/kg of iron, with the low end of the range associated primarily with gastrointestinal irritation while systemic toxicity occurs at the high end (IOM, 2001) and dose between 10 and 20 mg Fe/kg are regarded as non toxic to humans (Schumann, 2001). The dose of iron sulfate used in this study was in the higher limit of this range in rat model.

Our experiments showed that treatment with acute ferrous sulfate \((200 \text{ mgFe/kg})\) significantly induced an increase in the number of chromosomal aberrations in the bone marrow cells of rats \((P<0.001)\). Bone marrow cells are extremely susceptible to oxidative damage and sensitive to clastogenic chemicals (Umegaki et. al., 1997). In contrast, of the seven doses of TQ, the highest 21 and 24 mg/kg appeared to be less efficient in decreasing the total number of chromosomal type aberrations in animals treated with iron sulfate 200 mg/kg. These results are in line with previous reports which showed that high doses of TQ cause depletion of cellular glutathione (Badary et. al., 1998). Furthermore, TQ caused dose dependent genotoxic effects in Balb/c liver and kidney; it develops an increase of the frequency of chromosomal aberration and the DNA damage index. The increase of CAT is significant at 80 mg/kg. Similarly, the increase of the DNA damage index is significant at the highest concentration used \((80 \text{ mg/kg})\) (Harzallah et. al., 2012).
It is acknowledged that an increase in the frequency of chromosomal aberrations in bone marrow cells and consequently in peripheral blood lymphocytes is associated with an increased overall risk of cancer (Hagmar et. al., 1994). Most of the chromosomal aberrations observed in the cells are lethal, but there are many other aberrations that are viable and cause genetic effects, either somatic or inherited (Swierenga et. al., 1991). However in the present investigation a significant reduction was observed with pre, simultaneous and post treatment of TQ at the doses of 6, 9, 12, 15 and 18 mg/kg but TQ was found statistically most efficient in reducing the chromosomal aberrations at the dose of 18 mg/kg during simultaneous treatment. Although in one study, the best response in reducing serum levels of hepatic enzymes was observed at the dose of 9 mg/kg. Higher doses of TQ (18 mg/kg), however, provided lesser hepatoprotection (Nili-Ahmadabadi et. al., 2011). Many studies reported cytoprotective activity of TQ against the selective tissue toxicity induced by standard chemotherapeutic agents (Al-Shabanah et. al., 1998; Badary, 1999; Badary et al., 1997, 2000). It is likely that the chemopreventive properties of TQ may be related to its antioxidant activity (Houghton et. al., 1995; Burits and Bucar, 2000). Comet assay results also showed that, the increased TM induced by iron sulfate at 200 mg Fe/kg could be reduced by pre, simultaneous and post treatment with TQ at the doses of 6, 9, 12, 15 and 18 mg/kg but significant decrease in DNA damage in terms of decreased TM was observed at 18 mg/kg dose of TQ in comparison with negative control ($P<0.001$). On the other hand, pre, simultaneous and post treatment with TQ at the doses of 6, 9, 12, 15 and 18 mg/kg decreased OTM induced by Iron sulfate at 200 mg Fe/kg, but significant decrease in the OTM was observed at 18 mg/kg dose of TQ in comparison with negative control ($P<0.001$). Another study reported that with a dose of 5 mg/kg, TQ had a potent preventive effect on calculus formation and a highly disruptive effect on CaOx kidney calculi. They also tried 10 mg/ kg of TQ and documented its potent preventive effect on kidney calculus formation and a moderate effect on disruption of the kidney calculi. These data suggest that lower doses of TQ might be more effective on the treatment of CaOx kidney calculi. This is in accordance with our findings. Intramuscular administration of TQ into rats in doses of 4 mg/kg/day for four days stimulated the excretion of uric acid in urine. Furthermore, oral administration of TQ to both rats and mice in doses of 4-5 mg/kg for several days protected the animals against cisplatin induced nephrotoxicity (Badary et. al., 1997). In another study, Marozzi et. al. (1970)
reported a TQ-induced enhancement of histamine in induction of gastric ulcers, yet Kanter et al. (2005) reported that oral administration of TQ at a dose of 10 mg/kg one hour before oral absolute alcohol at a dose of 4 ml/kg to rats protected the animals against alcohol-induced ulcers by approximately 38% via antioxidant mechanisms that involved inhibition of reactive oxygen radicals and an increase in superoxide dismutase availability. In this respect the effect of TQ was less than that of the whole volatile oil. Present investigation demonstrated that at the dose of 21 and 24 mg/kg TQ could not show protective role against DNA damage in terms of TM and OTM in all treatments, which may be assigned to its pro-oxidant property at higher doses. In a study on the effect of TQ on lipid profile of rats, Bamosa and colleagues tested intraperitoneal injection of TQ with varying doses of 0.4 mg/kg to 8 mg/kg. They found that there was no linear association of the dose and lowering effect of TQ on serum lipids. The highest dose they used (8 mg/kg) had toxic effects (Bamosa et al., 2002). Recently, Badary et al. (2003) reported that the antioxidant activity of TQ is mainly due to its potent superoxide scavenging potential. It seems reasonable to postulate that TQ exerts its anticlastogenic activity by suppressing ROS generated during the metabolic activity of iron sulfate and hence protects against DNA damage.

TQ has been found more effective in protection against iron-induced free radicals via increased resistance to oxidative stress as well as the ability to reduce ROS production at the dose of 18 mg/kg during simultaneous treatment. Based on the broken line regression analysis of chromosomal aberrations, micronucleus and DNA damage data; it appears that the optimum level of TQ at which it shows its maximum protective effects against genotoxicity induced by iron sulfate is 18 mg/kg.

From the above discussion it is clearly stated that iron has the potential to cause significant oxidative damage leading to mutagenesis (Loeb et al., 1988). The precise mechanism of iron induced oxidative damage to DNA is not known but is believed to involve free iron that catalyzes the formation of hydroxyl radicals by the Fenton reaction (Nair et al., 1999). DNA damage may result directly by the oxidation of nucleoside bases or indirectly via the formation of lipid peroxides. To mitigate such oxidative DNA damage, flavonoids have been identified as fulfilling most of the criteria to be considered as antioxidants: the flavonoids inhibit the enzymes responsible for O2⁻ production (Hanasaki et al., 1994; Ursini et al., 1994); the low redox potentials of flavonoids
thermodynamically allow them to reduce highly oxidising free radicals such as $O_2^-$, $RO^-$ and $HO^-$ (Buettner, 1993); and a number of flavonoids chelate trace metals (Pietta, 2000). Besides scavenging, flavonoids may stabilise free radicals by complexing with them (Shahidi and Wanasundara, 1992). In vitro and animal studies have confirmed that flavonoids possess anti-inflammatory, antioxidant, antiallergenic, hepato-protective, antithrombotic, antiviral and anticarcinogenic activities (Middleton et al., 2000).

We chose to study QCT because of its superior antioxidant effect among other flavonoids. An extensive amount of in vitro and in vivo animal research has focused on the antioxidant potential of QCT. (De Whalley et al., 1990; Skaper et al., 1997; Huk et al., 1998; Shoskes, 1998; Mojzis et al., 2001; Inal et al., 2002; Kahraman et al., 2003; Su et al., 2003; Meyers et al., 2008; Das et al., 2008; Seufi et al., 2009; Annapurna et al., 2009; Hwang et al., 2009; Lu et al., 2010; Park et al., 2010; Tota et al., 2010; Yao et al., 2010). Animal evidence suggests QCT’s antioxidant effects afford protection of the brain, heart, and other tissues against ischemia-reperfusion injury, toxic compounds, and other factors that can induce oxidative stress. Some animal studies have looked at the anti-tumor properties of QCT. In one study, mice were inoculated with ascites tumor cells and then treated intraperitoneally with either QCT or its glycoside, rutin. Animals treated daily with 40 mg/kg QCT had a 20- percent increase in life span, while those treated with 160 mg/kg rutin had a 50% increase in life span. If the rutin treatment was split into two 80 mg/kg treatments per day, the increase in life span became 94% (Ferry et al., 1996). Another animal study looked at the effect of QCT on mice bearing abdominal tumors derived from a human pharyngeal squamous cell carcinoma line. The mice were given a daily intraperitoneal injection of QCT. All doses tested (20, 200, 400, and 800 mg/kg) demonstrated significant inhibition of tumor growth (Yokoo and Kitamura, 1997). In vivo tests with mouse with 4T1 breast cancer cells showed that QCT suppressed tumor growth and prolonged survival. QCT enhanced therapeutic efficacy of doxorubicin and reduced toxic side effects. Another Chinese study by Shan and Wang of the Hebei Medical University, investigated the effects of QCT on the growth of the colon carcinoma cells and the regulation effect of QCT on the Wnt/beta-catenin signaling pathway (Shan et al., 2009). Furthermore, QCT is known as an excellent metal chelator. Recently, it was confirmed that both anti-radical and chelating effects are involved in the protective effect of QCT (Cheng and Breen, 2000). When QCT was administered orally,
it was poorly absorbed from the digestive tract and did not have a great influence on the organs (Murota and Terao, 2003). QCT is the major flavonoid in the human diet and its daily intake with foods is estimated to be 50-500 mg (Deschner et. al., 1991). The protective effect of QCT may also be accounted for, at least in part, by their ability to enhance the activity of a variety of detoxification enzymes and/or to shift the metabolic profile of carcinogens such that the intra concentration of the reactive metabolites is diminished. The precise mechanism of the protective action of QCT on iron mediated cytotoxicity is assumed to be due to the scavenging of superoxide anions that produce hydroxyl radicals via the Haber-Weiss reaction, or by the chelation of metal ions that are used to produce highly toxic hydroxyl radicals from H$_2$O$_2$ via the Fenton reaction. We thus set out to observe the optimum level of QCT, at which it exhibits maximum protection against free radical induced damages due to iron sulfate toxicity in rat bone marrow cells by using chromosomal aberrations, micronucleus assay and in whole blood cells of Wistar rats by using single cell gel electrophoresis for 24 h. The precise mechanism of the protective action of QCT on iron mediated cytotoxicity is due to the scavenging of superoxide anions that produce highly toxic hydroxyl radicals via the Haber-Weiss reaction. This is based on the fact that like other polyphenolics, the biological effects of QCT are generally attributed to its antioxidative activities in scavenging ROS and chelating irons. Chromosome aberrations induced by iron sulfate (200 mg/kg) decreased markedly in the pre-treatment, simultaneous and post-treatment with QCT, except the three high doses (625, 750 and 875 mg/kg doses of QCT). Like other antioxidant, QCT might have pro-oxidant activity, at least under some circumstances. Long-term feeding of QCT (20 mg/day) to Sprague-Dawley rats increased serum and liver alpha-tocopherol concentrations and significantly decreased malondialdehyde concentrations, but also significantly decreased GSH concentrations and glutathione reductase activity (Choi et. al., 2005). Our study also indicates that QCT show cytotoxicity as there is increase in chromosomal aberration at the three high doses. However, the cytotoxicity induced by iron sulfate was found to be ablated by QCT. This indicates that QCT itself can induce dose dependent genotoxic and cytotoxic effect at high doses but the iron sulfate induced damages were reduced by it. Several pharmacological and biological properties that may be beneficial to the health of humans, including among others antioxidant, anti-inflammatory, and anti-carcinogenic characteristics, have been ascribed to QCT (Middleton et. al., 2000); however, the
antioxidant and free-radical scavenging properties of QCT and similar compounds, which are generally regarded as positive functions, also have been linked to less desirable pro-oxidant activities (Metodiewa et al., 1999; Boots et al., 2003).

Unlike the positive results obtained in vitro and in vivo following intraperitoneal dosing, tested in vivo in animal oral administration studies, QCT has not demonstrated any genotoxic activities. The results from the current series of in vivo genotoxicity studies conform with previous investigations of potential in vivo genotoxicity, which demonstrated an absence of positive activity when various relevant endpoints (i.e., MN, chromosomal aberrations, sister chromatid exchange, UDS, and alkali-labile DNA damage) in somatic cells obtained from animals treated orally with QCT were compared with those from negative controls (MacGregor, 1979; Aeschbacher et al., 1982; Ishikawa et al., 1985; Ngomuo and Jones, 1996; Taj and Nagarajan, 1996; Cierniak et al., 2004). An abundance of in vitro and in vivo animal experiments have attempted to elucidate QCT’s effect in cancer. In vitro evidence indicates that QCT has a variety of anticancer mechanisms, including antioxidant, antiproliferative, pro-apoptotic, cell signaling effects, and growth factor suppression, as well as potential synergism with some chemotherapeutic agents (Yoshida et al., 1990; Scambia et al., 1992; Larocca et al., 1995; Singhal et al., 1995; Richter et al., 1999; ElAttar and Virji, 1999; Caltagirone et al., 2000; Zhang et al., 2000; Ranelletti et al., 2000; Haghiac and Walle, 2005; Braganhol et al., 2006; Dihal et al., 2006; Rosner et al., 2006; Granado-Serrano et al., 2006; Gulati et al., 2006; Conklin et al., 2007; Hung, 2007; Aalinkeel et al., 2008; Ramos and Aller, 2008; Choi et al., 2008; Galluzzo et al., 2009; Jeong et al., 2009; Jung et al., 2009; Kawahara et al., 2009; Borska et al., 2010; Linsalata et al., 2010; Olson et al., 2010; Vidya Priyadarsini et al., 2010). QCT has also been shown to inhibit the growth of cancer in vivo in animal experiments designed to promote tumor formation (Castillo et al., 1989; Deschner et al., 1991; Pereira et al., 1996; Matsukawa et al., 1997; Yang et al., 2000; Ferrer et al., 2005; Jin et al., 2006; Choi et al., 2006; Warren et al., 2009;). While most of the animal studies have shown a beneficial effect (especially in preventing colon tumorigenesis), a high dose of QCT did not prevent UVB-induced carcinogenesis. (Steerenberg et al., 1997) A modified QCT (QCT chalcone) and a pH-modified citrus pectin were reported to reduce the growth of solid primary tumors (Hayashi et al., 2000).
In the present study reported here, chromosome aberrations induced by iron sulfate (200 mg/kg) decreased markedly in the pre-treatment, simultaneous and post-treatment with QCT at the doses of 125, 250, 375 and 500 mg/kg. In agreement with previous reports the multiple protective activities of QCT arise from its strong antioxidant properties (Scambia et al., 1991; Elattar and Virji, 2000; Caltagirone et al., 2000; Aligianni et al., 2001). Its antioxidant effect was documented in many in vitro and in vivo experimental studies (Mojzis et al., 2001). It has been recently observed that QCT may behave as a cytotoxic agent and as a mutagen at much higher doses (Sahu and Washington, 1991). Thus, the ineffectiveness of QCT at higher doses has been attributed to its prooxidant activities (Yoshino et al., 1999; Metodiewa et al., 1999). In the present study we also observed these apparently contradictory activities of QCT that it can act as both antioxidant and prooxidant, depending on concentration and the source of the free radicals (Laughton et al., 1989; Sugihar et al., 1999). In one study, protection was more prominent in 200 mg/kg as compared to 100 mg/kg. This protection might be due to the quenching of lipid peroxides by QCT from the cells. QCT also donates an electron to the free radicals formed in the body (Molina et al., 2003). Among pre, simultaneous and post treatments, the protective effect of QCT was most prominent in the simultaneous treatment and the best response was observed at 500 mg/kg. Pre, simultaneous and post treatments of QCT reduced the number of MNPCEs induced by iron sulfate at the doses of 125, 250, 375 and 500 mg/kg. The results suggest that QCT reduced the number of MNPCEs induced by iron sulfate, but it could not completely protect cells from damage. The most efficient anticlastogenic effect of QCT was observed in 500 mg/kg dose, at which it shows maximum protective effect against genotoxicity and clastogenicity induced by iron sulfate.

We observed significantly lower effect for both DNA damage in terms of tail moment and olive tail moment when QCT was administered through the i.p. route at the doses of 625, 750 and 875 mg/kg. In contrary to the present study another animal study looked at the effect of QCT on mice bearing abdominal tumors derived from a human pharyngeal squamous cell carcinoma line. The mice were given a daily intraperitoneal injection of QCT. All doses tested (20-, 200-, 400-, and 800 mg/kg) demonstrated significant inhibition of tumor growth. The 20 mg/kg dose had an effect only slightly less than that seen with 800 mg/kg. The effect on the growth of normal human fibroblast cells was
minimal. The author concluded that QCT appears to be a selective inhibitor of tumor cell growth (Castillo et. al., 1989). An effectively significant protection by pre, simultaneous and post treatment of QCT against the formation of oxidative DNA damage generated by iron sulfate was observed at the doses of 125, 250, 375 and 500 mg/kg. Two animal studies have looked at the anti-tumor properties of QCT. In one study, mice were inoculated with ascites tumor cells and then treated intraperitoneally with either QCT or its glycoside, rutin. Animals treated daily with 40 mg/kg QCT had a 20-percent increase in life span, while those treated with 160 mg/kg rutin had a 50-percent increase in life span. If the rutin treatment was split into two 80 mg/kg treatments per day, the increase in life span became 94 percent. These *in vivo* results are interesting since *in vitro* work showed rutin to have little effect compared to QCT on tumor tissue (Kuo, 1996; Larocca et. al., 1991). Human studies have not shown any adverse effects associated with oral administration of QCT in a single dose of up to four grams or after one month of 500 mg twice daily (Shoskes et. al., 1999). QCT may also have anti-mutagenic properties. A group of scientists lead by Gupta of the National Institute of Pharmaceutical Education and Research, Mohali, India, found that QCT may be a potential candidate as chemoprotectant. They came to this conclusion after treating rats, which were exposed to the hepatocarcinogen diethylnitrosamine (found in tobacco smoke and processed meat) with QCT. The hepatocarcinogen increased malondialdehyde and decreased glutathione levels in the liver, and increased plasma levels of aspartate transaminase and alanine transaminase. Treatment of the rats with QCT restored these levels and also reduced diethylnitrosamine induced DNA damage and apoptosis (Gupta et. al., 2009). Protection against H₂O₂ was confirmed for myricetin, QCT and rutin in Caco-2 and HepG2 cells (O’Brien et. al., 2000) and for QCT and luteolin in murine and human leukaemia cell lines (Horvathova et. al., 2003; 2004). With regard to other genotoxicants, QCT and rutin displayed antigenotoxic effects on DNA damage induced by mitomycin C, in a concentration-dependent manner (Undeger et. al., 2004). This study confirmed the antigenotoxic and protective effect of QCT and the best result was observed at the dose of 500 mg/kg QCT in simultaneous treatment. However, the mild prooxidant activity of QCT was observed at the high doses (625, 750 and 875 mg/kg). One possible mechanism for the protection against cyto- and genotoxicity may be that simultaneous treatment with QCT would allow interception of free radicals generated by iron sulfate before they reach DNA and induce cyto- and genotoxicity. In the present study we found QCT as a
strong inhibitor of iron-dependent OH· formation which helped in protecting against OH− induced chromosomal aberrations, MNPCEs and DNA damage in bone marrow and blood cells of Wistar rats. Further studies are required to elucidate the real nature and mechanism of the interactions of iron and QCT.

The protective action of TQ and QCT observed here suggests that they have antioxidative properties and damage induced by free radicals generated during the metabolic activity of iron sulfate in bone marrow and blood cells of rats was significantly ameliorated. TQ and QCT have been found more effective in protection against iron-induced free radicals via increased resistance to oxidative stress as well as the ability to reduce ROS production at the dose of 18 mg/kg and 500 mg/kg during simultaneous treatment.

Iron sulfate is a common chemical that is present in foods and beverages and that is used to treat iron deficiency anemia (Nelson and Cox, 2002). Despite this, in humans, several health problems have been related to high Fe intake (Chau et al., 1993), and as found in this study, it can also cause genotoxic damage. Based on these facts, the intake of this metal must be very carefully regulated.

Based on the broken line regression analysis of chromosomal aberrations, micronucleus and DNA damage data; it appears that the optimum levels of TQ and QCT at which they show their maximum protective effects against genotoxicity induced by iron sulfate are 18 mg/kg and 500 mg/kg.

Results of bone marrow chromosomal aberration assay, micronucleus assay and single cell gel electrophoretic assay (Comet assay), showed that TQ at 18 mg/kg and QCT at 500 mg/kg dose significantly decreased chromosomal aberrations, number of micronuclei and DNA damage caused by iron sulfate. The study provides evidence that TQ and QCT inhibit in vivo genotoxicity of iron sulfate in rats. Meanwhile, efforts should continue focusing laboratory research to gain further in-depth understanding of the antioxidant property of these nature endowed compounds for therapeutic uses in humans.