The present thesis entitled “Assessment of inhibitory potential of some naturally occurring agents in chemically induced renal toxicity and carcinogenesis” comprises of nine chapters, and bibliography. The main emphasis of this thesis is on the evaluation of antioxidant, anti-inflammatory and therapeutic potential of naturally occurring plant products and their constituents namely B.monnieri, Q.infectoria, caffeic acid, ellagic acid, chrysin and diosmin, against nephrotoxicity induced by different renal toxicants like Fe-NTA, TCE, Cyclophosphamide and KBrO₃ in animal models of renal toxicity. Role of chrysin and Q.infectoria was evaluated against DEN initiated and Fe-NTA promoted 2-stage renal carcinogenesis model in Wistar Rats.

The first chapter (Chapter I) deals with review of literature, we have discussed about basic aspects of cancer its types, properties of cancer cell, renal carcinogenesis, stages of renal carcinogenesis, risk factors associated with renal cancer, role of ROS, apoptosis and inflammation in cancer, chemoprevention, and chemopreventive agent. The second chapter (Chapter II) deals with methodology used to perform different parameters viz biochemical, histopathological, molecular and immunohistochemical.

In third chapter (Chapter III) we have discussed the effect of caffeic acid on Fe-NTA induced renal toxicity. Iron nitritotriacetate (Fe-NTA), a chief environmental pollutant, is known for its extensive toxic manifestations on renal system. In the present study caffeic acid, one of the most frequently occurring phenolic acids in fruits, grains and dietary supplements was evaluated for its shielding effect against the Fe-NTA induced oxidative, inflammatory and pathological damage in kidney. Fe-NTA was administered (9 mg Fe/kg body weight) intraperitoneally to the wistar male rats on 20th days while caffeic acid was administered orally (20 and 40 mg/kg body weight) prior to administration of Fe-NTA. The intraperitoneal administration of Fe-NTA (9 mg Fe/kg body weight) enhanced lipid peroxidation, xanthine oxidase and hydrogen peroxide generation with reduction in renal glutathione content, antioxidant enzymes, viz., catalase, glutathione peroxidase, glutathione reductase. A sharp elevation in the levels of myloperoxidase, blood urea nitrogen and serum creatinine has also been observed. Tumor promotion markers viz., ornithine decarboxylase (ODC) and [³H] thymidine incorporation into renal DNA were also significantly increased. Treatment of rats orally with caffeic acid (20 mg/kg body
weight and 40 mg/kg body weight) resulted in a significant decrease in xanthine oxidase (P<0.001), lipid peroxidation (P<0.001), γ-glutamyl transpeptidase (P<0.01) and H2O2 (P<0.01). There was significant recovery of renal glutathione content (P<0.001) and antioxidant enzymes (P<0.001). There was also reversal in the enhancement of renal ODC activity, DNA synthesis, blood urea nitrogen and serum creatinine (P<0.001). All these changes were supported by histological observations. The results indicate that caffeic acid may be beneficial in ameliorating the Fe NTA induced oxidative damage and tumor promotion in the kidney of rats.

In chapter 4 (Chapter IV), we have assessed nephrotoxicity and genotoxicity of cyclophosphamide an anticancer drug and its amelioration by ellagic acid. Cyclophosphamide (CPM), an alkylating agent is used as an immunosuppressant in rheumatoid arthritis and in the treatment of several cancers as well. In present study, Ellagic acid (EA), a naturally occurring plant polyphenol, was evaluated for its antigenotoxicity and antioxidant efficacy against the CPM-induced renal oxidative stress and genotoxicity in Swiss albino mice. The mice were given a prophylactic treatment of EA orally at a dose of 50 and 100 mg/kg body weight for 7 consecutive days before the administration of a single intraperitoneal injection of CPM at 50 mg/kg body weight. The modulatory effects of EA on CPM-induced nephrotoxicity and genotoxicity were investigated by assaying oxidative stress biomarkers, serum kidney toxicity markers, DNA fragmentation, alkaline unwinding, micronuclei assay, and by histopathological examination of kidney tissue. A single intraperitoneal administration of CPM in mice increase malondialdehyde (MDA) level with depletion in glutathione content, antioxidant enzymes activities viz. glutathione peroxidase, glutathione reductase, catalase, quinone reductase, induced DNA strand breaks and micronuclei induction. EA oral administration at both doses caused significant reduction in their levels, restoration in the activities of antioxidant enzymes, reduction in micronuclei formation, and DNA fragmentation. Serum toxicity marker enzymes like BUN, creatinine and LDH were also increased after CPM treatment and EA treatment significantly decreased these elevated levels. Present findings suggest a prominent role of EA against CPM induced renal injury, DNA damage and genotoxicity. Chapter V deals with the chemopreventive
Chapter 9  
Summary & conclusions

Efficacy of diosmin against TCE induced nephrotoxicity and its role in apoptosis. Diosmin (DM) is a naturally occurring flavone and has been found to possess numerous therapeutic properties. In this study, we used DM as a protective agent against the nephrotoxic effects of environmental toxicant Trichloroethylene (TCE). Rats were given oral treatment of DM at doses of 20 and 40 mg/kg body weight for 20 consecutive days along with the co-administration of TCE at 1000 mg/kg body weight. The protective effects of DM on TCE-induced oxidative stress and caspase dependent apoptosis were investigated by assaying oxidative stress biomarkers, lipid peroxidation, serum toxicity markers, alkaline unwinding assay, Caspases 3,7 and 9, BAX and p53 expression. Oral administration of TCE (1000 mg/kg body weight) in rats enhanced renal lipid peroxidation; depleted glutathione content and anti-oxidant enzymes; induced DNA strand breaks (p<0.001); modulated expression of BAX and p53 protein induced the expression of caspases 3, 7 and 9. Co-treatment with DM prevented oxidative stress by restoring the levels of antioxidant enzymes, further a significant dose-dependent decrease in DNA disintegration, and the kidney toxicity markers BUN (P<0.001), creatinine (P<0.01), and LDH (P<0.001) was observed. DM also effectively decreased the TCE induced up-regulation of BAX and p53. Thus, data from this study establishes the protective role of Diosmin against TCE induced renal damage.

In Chapter 6 (Chapter VI) Chrysin: a naturally occurring flavone was studied for its chemopreventive effect in chemically induced renal toxicity and carcinogenesis. Flavonoid family is a rich source of polyphenolic compounds and hence possess strong antioxidant and anti-inflammatory properties. The aim of this study was to determine the efficacy of chrysin; a bio-active flavonoid as an anticancer agent. Renal cancer was initiated by single i.p injection of DEN 200 mg/kg BW and promoted by twice weekly administration of Fe-NTA (mg Fe/Kg BW) for 16 wk. In the present study, we report the chemopreventive effects of chrysin against ferric nitritotriacetate (Fe-NTA) induced renal oxidative stress, Inflammation, hyperproliferative response, and two-stage renal carcinogenesis. To ascertain the molecular mechanism implicated in the antitumor promoting activity of chrysin, its effect was investigated on markers of tumor promotion and inflammation: ODC activity, PCNA, iNOS and COX-2 expression, and on levels of proinflammatory cytokines (IL-6, TNF-α, and PGE2). Pretreatment of animals with
Chrysin at both doses (20 and 40 mg/kg body weight) markedly inhibited all. Further, Fe-NTA enhances renal lipid peroxidation, with concomitant reduction in renal glutathione content (GSH), antioxidant enzymes, and phase II metabolizing enzymes. It induces serum toxicity markers viz, Blood urea nitrogen, creatinine and LDH. Prophylactic treatment of animals with chrysin before the administration of Fe-NTA was effective in modulating oxidative and renal injury markers and resulted in the diminution of Fe-NTA mediated injury. These results suggest chrysin as an effective chemopreventive agent having the capability to obstruct DEN initiated and Fe-NTA promoted renal cancer in the rat model.

Chapter VII deals with the role of *Bacopa monnieri* in amelioration of KBrO₃ induced nephrotoxicity and its possible role in tumor promotion and inflammation. *Bacopa monnieri* is a dietary herb used for neuroprotection and renal dysfunction in indigenous system of medicine in South Asia. Role of oxidative stress, inflammation and apoptosis has been reported in various diseased conditions including hyperproliferation and cancer. In the present study the wistar rats were subjected to prophylactic oral treatment of *B. monnieri* (100 and 200 mg/kg BW) against the nephrotoxicity induced by intraperitoneal administration of KBrO₃ (125mg/kg BW). Inhibitory potential of *B. monnieri* against chemically induced nephrotoxicity was evaluated in terms of increase activity of antioxidant enzymes, normalization of histopathological changes, ornithine decarboxylase and DNA synthesis inhibition, low expression levels of TNF-α, PGE₂, COX-2, NO and MPO as markers of inflammation. Caspase-3 and p53 were also studied. *B. monnieri* pretreatment was effective in preventing the damaging effects induced by KBrO₃. *B. monnieri* pretreatment also modulates NFκB protein expression levels. We found that the beneficial effect of *B. monnieri* pretreatment is mediated partially by its inhibitory potential on NFκB and TNF-α pathway mediated inflammation, caspase-3 and p53 mediated-tubular cell apoptosis, as well as by restoration of serum toxicity and histopathological changes against KBrO₃ administration.

Chapter VII discusses anticancer property of *Quercus Infectoria* in animal model of 2-stage renal carcinogenesis model. Ferric nitritotriacetate (Fe-NTA) is a well-established renal carcinogen. In this stud we have shown that *Quercus Infectoria* attenuates Fe-NTA
induced renal oxidative stress, hyperproliferative response and renal carcinogenesis in rats. Fe-NTA promoted DEN (N-diethyl nitrosamine) initiated renal carcinogenesis by increasing the percentage incidence of tumors and induces early tumor markers viz. ornithine decarboxylase (ODC) level and PCNA expression. Fe-NTA (9 mg Fe/kg body weight, intraperitoneally) also enhances renal Malondialdehyde, xanthine oxidase and hydrogen peroxide generation with reduction in renal glutathione content, antioxidant enzymes, viz., glutathione peroxidase, glutathione reductase, catalase, glucose-6-phosphate dehydrogenase and phase-II metabolizing enzymes such as glutathione-S-transferase and quinone reductase. It also enhances blood urea nitrogen and serum creatinine. Fe-NTA also lead to increase in levels of some inflammatory markers viz NO and MPO and some proinflammatory cytokines viz PGE-2 and TNF-α. Oral administration of rats with QI extract (75 and 150 mg/kg body weight) resulted in significant decrease in lipid peroxidation, xanthine oxidase, H₂O₂ generation, blood urea nitrogen (BUN), serum creatinine, renal ODC activity, PCNA expression and incidence of tumors. Renal glutathione content ($p < 0.01$), its metabolizing enzymes ($p < 0.001$) and antioxidant enzymes were also recovered to significant level ($p < 0.001$). Proinflammatory cytokines and inflammatory markers were also recovered to normal level by QI treatment. Thus, present study supports QI as a potent chemopreventive agent and suppresses Fe-NTA-induced renal carcinogenesis and oxidative and inflammatory response in Wistar rat.

In the last chapter, summary and conclusion of the work done is discussed. Results of each study are briefly described along with their significance. The results of our thesis supported by strong published literature reports suggest these natural compounds to be a potential candidate for prevention of chemically induced renal toxicity and carcinogenesis, since they restrain several biomarkers of oxidative stress, inflammation, apoptosis and tumor promotion in animal model. These properties make natural compounds studied pre-clinically useful. Further detailed mechanistic studies are necessary to divulge the beneficial effect of these naturally compounds before proceeding for clinical trials.