Summary & conclusion
Cancer is the prime cause of death in developed countries and the second major cause of death in developing world. The early diagnosis is very crucial for the effective treatment of cancer. But unfortunately, the lacuna in the treatment of cancer lies in the fact that its prognosis is not possible and diagnosis is poor. Despite massive effort to defeat cancer over the last few decades, the outcome of conventional strategies to combat cancer appears inadequate as the incidence and mortality of cancer is not reducing globally. Scientific community has given importance to the chemoprevention, an idea that appears to be a pragmatic and basic approach to fight cancer. Chemoprevention, a rapidly growing area of preventive oncology, deals with the prevention of cancer by one or more naturally occurring or synthetic compounds to block, reverse or prevent the process of carcinogenesis. Chemoprevention by phytochemicals is nowadays considered to be an inexpensive, readily applicable and accessible approach to cancer control and management. In addition, the promotion of awareness and consumption of phytochemicals as a cancer-preventive strategy would be cost-effective, within the health system.

Since chronic inflammation and over production of free radicals play vital role in various phases of carcinogenesis. Targeting aberrant inflammatory mediators is one of the best pragmatic approaches for chemoprevention. Further, induction of various endogenous anti-oxidants or detoxification enzymes by natural products will also give positive outcomes.

The present thesis entitled “Modulation of Inflammatory Biomarkers of Skin Carcinogenesis by Natural products in Swiss Albino Mice” comprises of seven chapters, summary & conclusion and bibliography.

Chapter III-V describes the preventive potential of natural products against 12-O-tetradecanoyl phorbol-13-acetate (TPA) - induced cutaneous oxidative stress, inflammation,
hyperproliferation and histological alterations, which are closely associated with the promotion phase of cancer development.

Chapter VI-VII includes the anti-tumorigenic potential of natural products against chemically induced skin carcinogenesis.

Chapter-I

Includes introduction and review of literature about the subject of research work.

Chapter-II

Describes in detail the materials and methods used to conduct the experiments of the present thesis.

Chapter-III

Describes the preventive effects of caffeic acid against TPA induced oxidative stress, inflammation and histological changes in mouse skin. Caffeic acid (3,4-dihydroxycinnamic acid), is one of the important phenolic acid present in medicinal plants, vegetables, beverages like wine, tea, coffee and apple juice. It is also an active constituent of bee propolis. It has a wide range of positive biological effects such as antioxidant, anti-inflammatory, immunomodulatory, anti-HIV, anti-tumor and anti-metastatic effects. Because over production of ROS and proinflammatory cytokine play important role in the promotion stage of carcinogenesis by transcriptional up-regulation NF-κB and COX-2. The present study was designed to investigate the protective effects of caffeic acid on TPA-induced oxidative stress, inflammatory responses, expression of NF-κB and COX-2 in mouse skin. The results show that caffeic acid significantly inhibited TPA-induced lipid peroxidation, xanthine oxidase activity (phase I enzyme), various inflammatory biomarkers and also found to up regulate GSH content and the activity of different phase II enzymes. Further, caffeic acid was found to inhibit TPA
induced cutaneous expression of NF-kB and COX-2. Histological findings further supported the protective effects of caffeic acid against TPA-induced cutaneous damage. Thus, present study demonstrate that pretreatment with caffeic acid has strong suppressing potential against TPA induced up-regulation of NF-kB and COX-2 via abrogation of oxidative stress, inflammatory responses and cytokine release. Therefore caffeic acid may prevent the early tumour promotional changes through its antioxidant and anti-inflammatory potential and its use may serve as one of the therapeutic approaches for cancer prevention.

Chapter IV

This chapter comprises the molecular mechanisms underlying the protective effects of geraniol against various inflammatory responses. Abnormal production of free radicals (ROS and RNS) and proinflammatory cytokines often act as trigger for the promotion stage of carcinogenesis via up-regulation of transcription factors and activation of MAP kinases. Geraniol, an important naturally occurring monoterpene, found in most of the herb oils and possesses multiple pharmacological activities. The present study was undertaken to investigate the protective effects of geraniol on TPA-induced oxidative stress, inflammatory responses, expression of p38MAPK, NF-kB, COX-2 and PCNA in mouse skin. Animals were divided into four groups I-IV (n=6). Group II and III received topical application of TPA at the dose of 10 nmol/0.2 ml of acetone/animal/day, for two days. Group III were pre-treated with geraniol (250 µg) topically 30 minutes prior to TPA administration. While group I and IV were given acetone (0.2 ml) and geraniol respectively. Geraniol significantly attenuated TPA-induced cutaneous lipid peroxidation, inflammatory responses and proinflammatory cytokines (TNF-α, IL-6, IL1-β) level. Geraniol also significantly modulated endogenous antioxidants, phase II metabolizing enzymes and histological alterations in mouse skin. Furthermore, geraniol suppresses expression of p38MAPK, NF-kB, COX-2 and PCNA
in mouse skin induced by TPA. Thus, present findings suggest that geraniol attenuates tumor promotional events, and could serve as one of the potential modality to prevent carcinogenesis.

Chapter-V

The basic aim of the present study is to investigate the effect of soy isoflavones (SIF) on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced cyclooxygenase-2 (COX-2) expression and hyper proliferation in mouse skin and to explore the underlying molecular mechanisms. It includes the assessment of modulatory effects of SIF against TPA induced oxidative stress, pro-inflammatory cytokines level, activation of NF-kB, expression of COX-2 and ki-67 in mouse skin. Animals were divided into five groups I-V (n=6). Group II, III and IV received topical application of TPA at the dose of 10 nmol/0.2 ml of acetone/animal/day, for two days. Animals of the group III and IV were pre-treated with SIF 1.0 μg (D 1) and 2.0 μg (D2) topically 30 minutes prior to each TPA administration. While group I and V were given acetone (0.2 ml) and SIF (D2) respectively. We have found that SIF pretreatment significantly inhibits TPA induced oxidative stress, proinflammatory cytokine production, activation of NF-kB, expression of COX-2 and ki-67. Histological findings further supported the protective effects of SIF against TPA-induced cutaneous damage. Thus, our results suggest that the inhibitory effects of SIF on TPA-induced NF-kB activation and COX-2 expression through modulation of proinflammatory cytokine production and oxidative stress. On the basis of the present findings we can assume that use of soy isoflavones can be a good promising chemopreventive agent.

Chapter-VI

This chapter provides substantial evidence that silibinin has strong anti-tumorigenic potential against chemically induced two-stage skin carcinogenesis. Silibinin is a major bioactive flavonolignan present in milk thistle (Silybum marianum) that possesses
antioxidant, anti-inflammatory and anti-carcinogenic potential and has been used as a traditional medicine for treatment of liver disorders. Topical application of TPA on the mouse skin is one of the well recognized models for the induction of oxidative stress, ROS production, cutaneous inflammation and hyperplasia. In light of the important roles of NF-kB, COX-2, iNOS, proinflammatory cytokines, VEGF and oxidative stress in carcinogenesis, chemopreventive efficacy of silibinin was studied in terms of cytoprotective enzymes activities, lipid peroxidation, redox status, inflammatory responses and the expression of various molecular markers in skin tissue. Topical application silibinin at dose of 9 mg/mouse effectively suppressed oxidative stress, deregulated activation of inflammatory mediators and tumorigenesis. Thus on the basis of the present findings it can be assume that, chemopreventive effects of silibinin were associated with up-regulation of different cytoprotective enzyme activities and down regulation of inflammatory mediators (NO, TNF-a, IL-6, IL-1β, COX-2, iNOS and NF-kB).

Chapter-VII

The purpose of this investigation is to study the preventive effects of quercetin on DMBA-initiated and TPA-promoted skin tumor development and against TPA-induced cutaneous oxidative stress, xenobiotic metabolizing enzymes, endogenous antioxidants, myeloperoxidase activity and epidermal expression of COX-2, iNOS, NF-kB in Swiss albino mice. The findings of this study revealed that topical application quercetin effectively suppressed chemically induced skin tumorigenesis, and this prevention is closely associated with the inhibition of TPA-induced lipid peroxidation, activation of NF-kB, COX-2, iNOS, elevated myeloperoxidase and xanthine oxidase (phase I enzyme) activity, depletion of both antioxidant and phase II enzymes. In addition, these results were further supported by the histopathological examination of skin.
For the tumor studies, 28 mice were divided into four groups of 7 mice each. The first group served as control and was treated topically on the dorsal skin with acetone. The tumorigenesis in the group II, III and IV was initiated by DMBA application and promoted with TPA. Mice in the group III and IV were treated with quercetin topically on the dorsal skin. Topical application of quercetin decreased skin tumor incidence in the group III and IV as compare to group II, suggesting its chemopreventive efficacy against DMBA-initiated and TPA promoted two-stage carcinogenesis.