CHAPTER 8
SUMMARY AND CONCLUSION

⇒ Phytochemical analysis of *Vitis vinifera* peel and seed aqueous extracts showed the presence of flavonoids, alkaloids, phytosterols and triterpenoids and the *in vitro* antioxidant activities may be attributed to the polyphenols by scavenging the free radicals.

⇒ The present study showed that high content of phenols, ascorbic acid and flavonoids were present in *Vitis vinifera* seed than in peel extracts.

⇒ The study was focused on synthesizing biocompatible gold nanoparticles through *Vitis vinifera* seeds and peels which was found to be ~50 nm in size coated with the polyphenols of the *Vitis vinifera* seeds and peels.

⇒ According to the IC$_{50}$ concentration evaluated through cytotoxicity assays, the concentration of the peel and seed gold nanoparticles and aqueous extracts to be used for assays were determined.

⇒ Gold nanoparticles showed toxicity in A431 skin cancer cell line, while it was non-toxic to normal human immortalized keratinocyte cell line (HaCaT) which substantiates that gold nanoparticles synthesized biologically would have showed chemotherapeutic activity.

⇒ This cytotoxicity of the gold nanoparticles may be attributed to the synergic effects of the phenolic moieties assumed to have anti-proliferative activities.

⇒ A431 cells when treated with peel and seed gold nanoparticles showed morphological changes as it entered the apoptosis pathway leading to secondary necrosis.

⇒ Gold nanoparticles were able to induce ROS in the skin cancer cells resulting in cellular damage and this can make impairment of mitochondrial electron transport chains.
The contact between gold nanoparticles and cells is believed to induce formation of reactive oxygen species (ROS) signaling cascades which controls inflammatory processes, cellular proliferation and cell death.

*Vitis vinifera* seed and peel gold nanoparticles treated cells showed greenish orange for propidium iodide and Annexin V-FITC positive cells which indicated that A431 cells has undergone apoptosis. The staining also confirmed the efficiency of bioconjugated gold nanoparticles causing apoptosis and secondary necrosis.

A431 cells treated with seed and peel gold nanoparticles revealed morphological features such as membrane blebbing, shrinkage of cell and chromatin condensation after 24 hrs treatment.

The present study also showed that *Vitis vinifera* peel and seed AuNPs were able to decrease membrane potential such that it follows mitochondrial pathway in the process of apoptosis which is considered as an important mediator of cell apoptosis.

The cells were able to internalize the gold nanoparticles and mount stress response in the nuclear level.

Gold nanoparticles of 52.2±6.0 and 55.1±5.1nm synthesized using peel and seed extracts of *Vitis vinifera* are capped with aromatic compounds, phenols, alkaloids, flavonoids and other polyphenols may or may not affect cell viability in short term but can affect cell proliferation and cause damage to DNA as the cell response was long lasting.

Immunostimulatory potential of the grape seed and peel AuNPs were studied in Swiss albino mice and was found to increase total WBC count and haemoglobin content significantly.

*Vitis vinifera* peel and seed AuNPs at 2.5 mg/kg body weight significantly increased the cell mediated immune response by stimulating the response of sensitized T-lymphocytes.

The gold nanoparticles with ~50 nm also showed significant increase in the antibody synthesis by inducing the humoral immune response.
Topical application of *Vitis vinifera* seed and peel gold nanoparticles (2.5mg/kg/b.wt./animal/day) (~50 nm) on the DMBA initiated and TPA promoted skin papillomagenesis in Swiss albino mice for 16 weeks revealed chemopreventive potential either by preventing or inhibiting the formation of tumors.

This study also suggested that *Vitis vinifera* peel and seed AuNPs and extracts suppressed abnormal skin cell proliferation occurring during DMBA-induced skin papillomagenesis and demonstrated significant chemopreventive potential of peel and seed gold nanoparticles in DMBA induced skin carcinogenesis than seed and peel aqueous extracts.

The gold nanoparticles acted either by penetrating the skin and inhibited the formation of papillomas or accumulated inside the tumors to prevent further progression of the growth of papillomas.

The beneficial action of *Vitis vinifera* peel and seed AuNPs is probably due to its ability to stimulate the antioxidant enzymes in the cells.

Topical application of *Vitis vinifera* peel and seed gold nanoparticles also reverted back the antioxidant level in the treated mice showing the antioxidant potential of the gold nanoparticles.

Phytochemicals from *Vitis vinifera* peel and seed extracts that formed the coating on gold nanoparticles may also have offered therapeutic potential to the cancer cells.

Treatment with gold nanoparticles restored the skin with normal histology through scavenging free radicals, balancing antioxidant enzymes and control over lipid peroxidation thus showing the therapeutic properties of gold nanoparticles biosynthesized using *Vitis vinifera* peel and seed.

Topical application of peel and seed gold nanoparticles for 16 weeks on DMBA induced skin papillomagenesis resulted in decreased expression of p53, BcL-2 and cytokeratins while DMBA application on the skin of the carcinogen control group resulted in upregulated expression of p53, Bcl-2 and cytokeratins.
 ⇒ *Vitis vinifera* peel and seed AuNPs showed better *in vitro* as well as *in vivo* anticancer activities when compared to aqueous extracts treated groups.

⇒ To conclude, our study suggests that *Vitis vinifera* seed and peel AuNPs possess chemopreventive potential against human epidermoid skin carcinoma cells (A431) *in vitro* and also against DMBA induced skin papillomagenesis *in vivo* with the mechanistic pathway of apoptosis in both *in vitro* and *in vivo*.

⇒ This can be due to the combined efficacy of gold nanoparticles in targeting the cancer cells and polyphenols that are capped on the nanoparticles provides chemopreventive effect on the cancer cells.

⇒ This study will lead to developing a combination of drug using nanoparticles and polyphenols and thus enhancing the efficacy of the particular drug and its further use as a chemopreventive agent.

⇒ Further studies has to be done to analyze the biodistribution of gold nanoparticles in skin tissues and its toxicity in the internal organs.

⇒ The gold nanoparticles on entering the living system can react with the proteins in the blood and can be possibly capped with proteins it encounters.

⇒ The gold nanoparticles can also react with the proteins present in the cell culture media and may possibly change the size of nanoparticles leading to agglomeration.

⇒ These drawbacks in this study may be rectified in further research works to be done.