CONCLUSION
Summary

The present study entitled “Analyzing the Chemopreventive Potential of Medicinal Plants in Cervical Cancer and to Study Underlying Molecular Mechanisms Governing the Anticancer Activity” includes in vitro experiments to elucidate the anti-cancer potential of aqueous extract of the bark of Cinnamon cassia and Ficus religiosa in cervical cancer cell lines. In addition, this study also includes in vitro and in vivo experiments with novel herbal formulation (FC) to elucidate its anti-cancer potential. We hypothesize that Cinnamomum (common and cassia) and Ficus religiosa alone as well as in combination possess anti-cancer activity and may target important signaling pathways and biological markers whose deregulation leads to cervical cancer.

Cervical cancer is a major woman health problem, with invasive cervical cancer being a leading cause of cancer death in women worldwide (Ferlay et al., 2008). It is the most common cancer among women in developing countries where 86% of the global cervical cancer burden of approximately 529,409 cases and 274,883 deaths are found annually (Bruni et al., 2014a). The effective cervical cancer screening programs, which can detect and treat the precancerous lesions at an early stage, have dramatically reduced the disease incidence as well as death (Dickinson et al., 2012). However, prognosis for the advanced cancer remains poor despite several efforts to improve treatment outcomes. For locally advanced disease, radiation is combined with low-dose chemotherapy; however, this modality often leads to severe toxicity (Kirwan et al., 2003). Considering this scenario of current treatment modalities, newer approaches of cancer management deserve immediate attention.

Traditional medicines have been used from time immemorial to treat various chronic ailments including cancer. Recently, complementary and alternative medicine (CAM), particularly herbal medicine, is becoming popular as an adjunct to chemotherapy
(Helyer et al., 2006). Plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted as one of the main sources of chemopreventive drugs (Cragg et al., 2005; Pandey et al., 2009). A wide variety of naturally occurring substances from plants have been shown to offer protection from carcinogenic exposure (Aziz et al., 2008). Various scientific studies support herbal medicines, an important part of traditional medicines, as potent anti-cancer drug candidates (Pandey et al., 2009).

In this contribution, we proposed to evaluate anti-cancer activity of the bark of Cinnamon (*Cinnamomum zeylanicum* and *Cinnamomum cassia*) and *Ficus religiosa* alone or in combination in cervical cancer. To test our hypothesis, we aimed at following objectives

1. To analyze the antineoplastic potential of Cinnamon in vitro in cervical cancer cell line
2. To analyze the antineoplastic potential of Ficus in vitro in cervical cancer cell lines
3. To develop a novel herbal formulation (FC) and test its antineoplastic potential in vitro in cervical cancer cells and *in vivo* in mouse melanoma model

**NOVEL FINDINGS OF THE STUDY**

➤ **The thesis for the first time demonstrates the following interesting findings with *Cinnamomum cassia* in SiHa**

- Cinnamon treatment alters growth kinetics the cells
- Cinnamon extract induces apoptosis in the cervical cancer cells through increase in intracellular calcium signaling as well as loss of mitochondrial membrane potential
- Cinnamon decreases cell migration through reduction in MMP-2 expression
Cinnamon significantly reduces the expression of Her-2 oncoprotein.

The thesis for the first time demonstrates the following interesting finding with Ficus religiosa in SiHa and HeLa cells:

- Ficus modulates the growth kinetics of cervical cancer cells
- Ficus induces cell cycle arrest and altered the expression of cell cycle regulating protein in SiHa
- Ficus induces apoptosis through increase in intracellular calcium and decrease in mitochondrial membrane potential in HeLa
- Ficus increases p53, caspase-3 and cytosolic cytochrom-c expression in HeLa
- Ficus decreases invasion and migration, and alters the expression of MMP-2 and HER-2 oncoproteins of SiHa and HeLa cells
- Ficus reduces the expression of viral oncoproteins E6 and E7

The thesis also for the first time developed a novel herbal formulation (FC) and demonstrated the following interesting in vitro and in vivo finding

- FC induces apoptosis in cervical cancer cells
- FC induces cell death involved early generation of Reactive Oxygen Species (ROS)
- FC causes robust increase in nitric oxide (NO) in parallel to ROS
- FC decreases mitochondrial membrane potential
- Oral administration of FC extract inhibits melanoma growth in vivo.
- FC stimulates Th1 cytokine expression in tumor-bearing mice
Implications

This study attempts to elucidate the anti-cancer potential of two plant bark materials namely *Cinnamomum cassia* and *Ficus religiosa* in cervical cancer cells by examining their effects at cellular and molecular level. Further, the study with *Cinnamomum cassia* throws light on mechanism by which ACE-c induced apoptosis and inhibited migration of the SiHa cells. The study with *Ficus religiosa* shows differential mechanism involved in regulation of anti-cancer activity by FR$_{aq}$ in SiHa and HeLa. The study also provides in vitro anti-cancer mechanism of novel herbal formulation (FC) in SiHa and HeLa cells. It has also established *in vivo* anti-cancer activity along with initial data on immunomodulatory activity in C57BL/6 mouse model. The novel information gathered from the current study clearly indicates the potential of *Cinnamon cassia* and *Ficus religiosa* and herbal formulation FC as phytomedicines for the treatment of cervical cancer.

Societal Relevance

Cervical cancer is the second most common malignancy worldwide and the leading cause of cancer related deaths among women in India. The failure of conventional chemotherapy to reduce mortality invites attention towards new alternative approaches that would reduce morbidity as well as side effects conferred by conventional chemotherapy. This study may provide a clue which would enable development of effective herbal interventions in the management and prevention of cervical cancer, and would also reduce the side effects of conventional therapy.
Future Perspectives

The herbal formulation developed from the above study could be tested in clinical trials in cervical cancer patients. This would further validate our experimental studies and would translate our findings from bench to bedside.