UGC Major Research Project Summary

Title of research project:

"Influence of Smokeless Tobacco on Cardiovascular risk and insulin resistance after menopause: Modulation by Sirtuin (SIRT) Receptor acting phytoestrogens."

File no.: 41-719/2012 (SR) (HRP) dated 1.7.2012

Name of the Principal Investigator:

Dr. (Mrs.) Anuradha S. Majumdar, Department of Pharmacology, Bombay College of Pharmacy, affiliated to University of Mumbai.

Summary:

Estrogens are steroid hormones which generate and regulate menstrual cycle, and known for its beneficial role on cardiovascular system (Esteva & Hortobagyi, 2006), carbohydrate metabolism (Song *et al.*, 2005) and lipid metabolism (Milewicz & Demissie, 2002; Moreira *et al.*, 2014) and inhibits expression of inflammatory markers (Maggio *et al.*, 2009) and maintains homeostasis (Milewicz & Demissie, 2002).

After birth several million follicles slowly declines to a few thousand as women approach menopause (Parihar, 2001). This denotes, more than 99% of ovarian follicles undergo degenerative changes, known as 'atresia'. The condition in which ovaries become depleted of primordial follicles is referred in medical literature as 'menopause'. As year passes changes in hormone production affects various parts of body in particular bones (osteoporosis), cardiovascular system (stroke and congestive heart failure) and the urogenital system (Majumdar & Nirwane, 2014). Hormone replacement therapy (HRT) has been found effective in alleviating the majority of symptoms of menopause. However, several clinical studies and meta-analysis has reported increased risk of stroke, sudden death, breast and endometrial hyperplasia which constraints use of HRT (Adlercreutz *et al.*, 1991; Mosca *et al.*, 2001; Ettinger *et al.*, 2012; Steinkellner *et al.*, 2012).

It has been projected that by the year 2030, tobacco use in the developing world is expected to lead to 7 million deaths annually (Öberg *et al.*, 2011). Smokeless tobacco use is a global problem impacting all regions. Smokeless tobacco use is more prevalent in countries of Asia, Africa and the Middle East than in Europe and the America. Smoking and tobacco use pose a serious health risk in women. It has been reported that women who smoke have an accelerated ovarian aging leading to early menopausal transition (McKinlay *et al.*, 1985; Guida *et al.*, 2012). Research demonstrates smokeless tobacco predispose the individual to various cardiovascular and metabolic aberrations such as diabetes mellitus (Gupta & Ray,

2003). There are reports that suggest that smoking in women results in early menopause (Guida *et al.*, 2012). So it was imperative to postulate that the post menopausal women consuming smokeless tobacco will be at higher risk to suffer from cardiovascular and metabolic complications.

The importance of plant derived estrogens or phytoestrogens has in recent times been increased by the realization of demerits of hormone replacement therapy (Hays *et al.*, 2003). Resveratrol has shown to be an antioxidant, cyclooxygenase inhibitor, peroxisome proliferator-activated receptor alpha activator, endothelial nitric oxide synthase inducer and silent mating type information regulation two homolog 1 (SIRT1) activator (Baur, 2010). Pterostilbene, a resveratrol relative is a dimethylated analog of resveratrol, is known to be effective in cancer, inflammation, dyslipidaemia, cognitive defects, cardiovascular and metabolic malfunctions (Maurya *et al.*, 1984; Rimando *et al.*, 2005; Pari & Satheesh, 2006; Joseph *et al.*, 2008; Remsberg *et al.*, 2008). There are dearth of data exploring the impact of smokeless tobacco consumption by women's and health adversities. This prompted us to investigate the detrimental outcomes of the use of smokeless tobacco in peri-menopausal and post menopausal state in appropriately designed studies in rodents. Further, we explored the natural nutraceutical regimen with resveratrol and pterostilbene in this milieu to find a possible alternative to chronic hormonal replacement therapy and could provide a viable benefit to treat diabetic and cardiovascular complications associated with smokeless tobacco in postmenopausal women (Majumdar *et al.*, 2013).

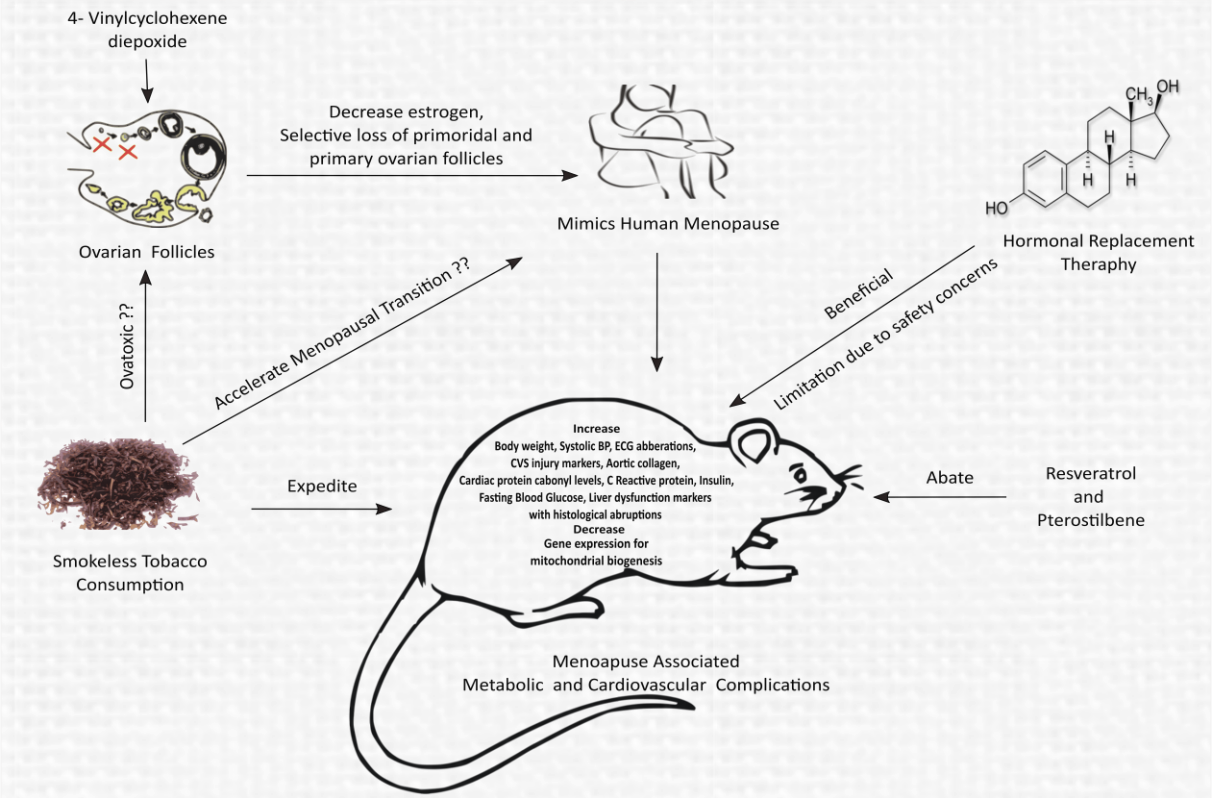

Figure.1 Hypothesis

The objective of this work was based on pharmacological evaluation of **nutraceutical**s for its potential against **smokeless tobacco** induced **cardiovascular and metabolic aberrations** in **estrogen deficient female rats**. We administered VCD (80 mg/kg) intraperitoneally for 30 days to induce estrogen deficiency states in 28 day old Sprague Dawley rats. We administered orally, aqueous extract of smokeless tobacco (AEST) for 60 days to the estrogen deficient female rats in a calculated amount (100 mg/kg) which simulates human exposure in rats. We evaluated the impact of AEST on trailing and profiling markers of metabolic derangements (*body weight, anthropometrical parameters, circulating markers of liver dysfunction, lipid profile, insulin homeostasis and histological evaluations of pancreas, liver and adipose tissue*), cardiovascular risk markers (*Systolic blood pressure, ECG, aortic collagen, cardiac carbonylated proteins, circulating markers of myocardial injury, C reactive protein, serum estradiol levels and histological evaluations of cardiac tissue and ovaries*) and mRNA expression studies (*hepatic, gastrocnemius muscles and cardiac gene expression of sirtuin-1, peroxisome proliferator-activated receptor-γ coactivator-1α,, peroxisome proliferator-activated receptor-α, nuclear respiratory factor-1, mitochondrial-transcription-factor-A and mitochondrial content*) in estrogen deficient states. Furthermore, we evaluated

the impact of nutraceuticals, resveratrol and pterostilbene at various doses in these perturbations. In addition, plasma nicotine and cotinine levels were also quantified by HPLC.

Results and conclusion

We simulated the human blood nicotine levels. Our result showed the impaired cardiovascular health, glucose tolerance, insulin and lipid homeostasis, linked to defective mitochondrial biogenesis in estrogen deficient states. The 60 days oral exposure to aqueous extract of smokeless tobacco greatly expedited the above outcomes in this estrogen deficient state. The resveratrol (5 mg/kg, 20 mg/kg and 40 mg/kg), pterostilbene (5 mg/kg, 20 mg/kg and 40 mg/kg) *per se* and combination therapy (resveratrol 20 mg/kg + pterostilbene 20 mg/kg) counters the smokeless tobacco induced metabolic and cardiovascular aberrations through mitochondrial biogenesis by upregulating the genes of energy metabolism in high energetic tissues (heart, skeletal muscle and liver).

Future Prospective

The ever increasing number of postmenopausal women worldwide and the **pervasive use of smokeless tobacco** among women are plausible to deteriorate the overall health. Our result substantiates and generates compelling preclinical evidence about the detrimental outcomes of smokeless tobacco on **cardiovascular and metabolic parameters** in **experimental peri-menopausal and menopausal states** in female rats. Moreover, the fact that metabolic and cardiovascular diseases involves cluster of anomalies hence, targeting at multiple points may exhibit better therapeutic approach than single target modulation. In agreement the syndicate of the nutraceuticals such as, resveratrol and pterostilbene showed a way for much promising future therapies in alleviating cardiovascular and metabolic complications. Importantly, there will be a hope that these **nutraceuticals may sideline the controversial hormonal therapies** thus offering safe treatment options.

Acknowledgement

This work was supported by the major research project under University Grant Commission (UGC letter no. F. No. 41-719/2012 (SR) dated. 23/07/2012), New Delhi, India.

Output of study

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Name of the Principal Investigator:

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Output of the study:

1. Manpower trained:

Mr. Abhijit Mahavir Nirwane was appointed as project fellow under this UGC major research project (File no. 41-719/2012 (SR) (HRP) dated 1.7.2012) on 17 October 2012. During the three years tenure of this project he learnt various techniques such as real-time PCR, radioimmunoassay, pharmacokinetic studies using HPLC etc. which was the necessary requirements for the completion of this project. Additionally, he learnt stereotaxic surgery in rats and standardized laboratory assays for estimation of different biochemical markers in biological fluids and tissues.

2. PhD awarded:

With the compilation of this UGC major research project (File no. 41-719/2012 (SR) (HRP) dated 1.7.2012) the PhD will be awarded to Mr. Abhijit Mahavir Nirwane who was registered as a PhD student in University of Mumbai (354/30-04-2013) and worked as a project fellow under this UGC major research project for the tenure from 17 October 2012 to 1 July 2015.

3. Publications of results:

The manuscript related to the current project were communicated to the following journals,

1. Resveratrol and pterostilbene improves glucose tolerance, insulin homeostasis and mitochondrial biogenesis in smokeless tobacco associated metabolic derangements in estrogen deficient female rats. Journal of Functional Foods (Accepted 2016).

2. Resveratrol and pterostilbene attenuates the smokeless tobacco induced cardiovascular aberrations in estrogen deficient female rats by fostering the genes of energy metabolism. Food and Chemical Toxicology (Communicated).
3. Majumdar AS, Nirwane AM. Adverse Impact of Smokeless Tobacco in Precipitating Metabolic and Cardiovascular Anomalies in Estrogen Deficient States. Austin J Pharmacology Therapeutics 2014; 2(7):1-3. (Letter to editor).
4. Accepted Abstract (Abstract ID-AM-54) entitled "Resveratrol improves lipid profile, insulin homeostasis and mitochondrial biogenesis in smokeless tobacco associated metabolic derangements in estrogen deficient rats: Implication of SIRT1 and PGC-1α pathway" for poster presentation in 2015 AAPS Annual meeting and Exhibition, going to be held at orange county convention center, Orlando, Florida, USA.
5. Accepted Abstract (Abstract ID-AM-28) entitled "Pterostilbene attenuates smokeless tobacco induced cardiovascular aberrations in estrogen deficient female rats through fostering the genes of energy metabolism" for poster presentation in 2015 AAPS Annual meeting and Exhibition, going to be held at orange county convention center, Orlando, Florida, USA.

4. Impact of work:

The ever increasing number of postmenopausal women worldwide and the pervasive use of smokeless tobacco among women are plausible causes for deteriorating overall health in older women. There is a great need to unravel these precipitating life style factors causing health adversities in post menopausal women.

This investigation proves conclusively the greater risk of adverse cardiovascular and metabolic perturbations upon smokeless tobacco administration in experimental menopausal states in female rats. The conventional hormonal therapy may offer benefits but lacks safety considering its high risk to benefit ratio. This preclinical study provides insights on the benefits of (trans- 3,4',5-trihydroxystilbene) and pterostilbene (trans- 3, 5-dimethoxy- 4'-hydroxystilbene) treatments in eliciting cardiovascular and metabolic benefits in estrogen deficient states with added liability of smokeless tobacco exposure on females. Moreover, the syndicate of resveratrol and pterostilbene showed more promising results in achieving these than their *per se* administration. These benefits were possibly achieved through activation of SIRT1-PGC-1α-PPAR-α axis which has been proven to be the cornerstones in mitochondrial biogenesis imparting improved energy homeostasis.

The data generated from this preclinical study,

1. Profiles the impact of AEST.
2. Promises the benefits of dual treatment with the stilibenes, Resveratrol and Pterostilbene providing a viable alternative to HRT.
3. Generated the empirical preclinical proof substantiating the impact of smokeless tobacco as a major risk in peri-menopause and menopause states in females.

There is a need to conduct clinical studies to provide clinical proof for this concept. It will be pertinent to test this hypothesis in the postmenopausal women consuming smokeless tobacco products and compare with HRT outcomes. There is need to further probe the SIRT1, AMPK and PGC-1α cross talk in this mileau. It is important to develop selective activators of these targets to bolster energy homeostasis and cardiovascular health.

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