" Studies on the Potential Chemomodulatory Effects of a Honey Bee Product in Prostate Cells "

A thesis submitted for the partial fulfillment of the requirements of Ph.D. Degree in Pharmaceutical sciences (Pharmacology and Toxicology)

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وَأَوْحَى رَبكَ إِلَى النَّحْلِ أَنِ اتَّخِذِي مِنَ الْجِبَالِ بُيُوتًا وَمِنَ الشَّجَرِو َمِمَّا يَعْرِشُون(٦٨) ثُمَّ كُلِي مِن كُلِّ الثَّمَرَاتِ فَاسْلُكِي سُبُلَ رَبِّكِ ذُلُلاً يَخْرُجُ مِن بُطُونِهَا شَرَابٌ مُّخْتَلِفٌ أَلْوَانُهُ فِيهِ شِفَاء لِلنَّاسِ إِنَّ فِي ذَلِكَ لآيَةً لِّقَوْمٍ يَتَفَكَّرُونَ (٦٩) سورة النحل

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"I dedicate this thesis to

my dear parents. Without their support, patience, understanding and love, the completion of this work wouldn't have been possible"

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Abstract

Evidence is growing for the beneficial role of selective estrogen receptor modulators (SERM) in prostate diseases. Caffeic acid phenethyl ester (CAPE) is a promising component of propolis that possesses SERM activity. The current study aimed at investigating the modulatory impact of CAPE on docetaxel (DOC) and paclitaxel (PTX) cytotoxicity in prostate cancer (PC) cells and exploring the possible underlying mechanisms for this chemomodulation. CAPE significantly increased DOC and PTX potency in PC-3, DU-145 and LNCaP PC cells. Combination index calculations showed synergistic interaction of CAPE/DOC and CAPE/PTX co-treatments in all the tested cell lines. Subsequent mechanistic studies in PC-3 cells indicated that cyclin D1 and c-myc were significantly reduced in the combined treatment groups with concurrent increase in $p27^{kip}$. DNA-ploidy analysis indicated a significant increase in the percentage of cells in pre-G1 in CAPE/DOC and CAPE/PTX co-treatments. Decreased Bcl-2/Bax ratio together with increased caspase-3 activity and protein abundance were observed in the same groups. Estrogen receptor-β (ER-β) and its downstream tumor suppressor forkhead box O1 (*FOXO-1*) levels were significantly elevated in CAPE and combination groups compared to DOC or PTX-alone. ER-α and insulin like growth factor-1 receptor (IGF-1R) protein abundance were reduced in the same groups. CAPE significantly reduced AKT, ERK and ER-α (Ser-167) phosphorylation in PC-3

cells. CAPE-induced inhibition of AKT phosphorylation was more prominent (1.7 folds higher) in cells expressing ER-α such as PC-3 compared to LNCaP. In conclusion, CAPE enhances the antiproliferative and cytotoxic effects of DOC and PTX in PC cells. This can be, at least partly, attributed to CAPE augmentation of DOC and PTX pro-apoptotic effects in addition to CAPE-induced alterations in ER-α and ER-β abundance.

List of Abbreviations

α-ERKO	ER- β knockout transgenic mice
AD	Alzeheimer's disease
AKT	Protein kinase B
ATRA	All-trans retinoic acid
AV	Atrio-ventricular
β-ERKO	ER- β knockout transgenic mice
BPH	Benign prostate hyperplasia
CAPE	Caffeic acid phenethyl ester
CAT	Catalase
CI	Combination index
COX-2	Cyclo-oxygenase-2
CRPC	Castration-resistant prostate cancer
CYP19	Aromatase
DAB	3,3'-diaminobenzidine
DEN	Diethylnitrosamine
DES	Diethylstilbestrol
DHT	Dihydrotestosterone
DMF	Dimethyl formaldehyde
DMSO	Dimethyl sulfoxide
DOC	Docetaxel
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DRI	Dose reduction index
E2	Estradiol, Estrogens
ER- β	Estrogen receptor β
ER-α	Estrogen receptor α
ERK	p44/42 Mitogen activated protein kinase (MAPK)
FACS	Flourescence activated cell sorting (Flow cytometry)
FCAPE	Catechol-ring fluorinated derivative of CAPE
FOXO-1	Forkhead box O1
GPx	Glutathione peroxidase
GSH	Reduced glutathione
HO-1	Heme oxygenase-1
IC50	Half-maximal inhibitory concentration
ICAM-1	Intercellular adhesion molecule 1
IFN-γ	Interferon-gamma
IGF-1R	Insulin like growth factor-1 receptor
Ig-E	Immunoglobulin-E
IL-6	Interleukin -6

iNOS	Nitric oxide synthase
KLF5	Krüppel-like zink finger transcription factor5
LHRH	Luteinizing hormone resleasing hormone
LOX	5-Lipoxygenase
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein
MDA	Malone dialdehyde
MPO	Myeloperoxidase
MPT	Mitochondrial permeability transition
NF-kB	Nuclear factor (NF)-kB
PARP	Poly(ADP-ribose) polymerase
PBS	Phosphate buffesred saline
PC	Prostate cancer
PI	Propidium iodide
PI3K	Phosphoinositide-3-kinase
PIN	Prostatic intraepithelial neoplasia
pNA	p-nitroaniline
PSA	Prostate-specific antigen
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PTX	Paclitaxel
RAR-α	Retinoic acid receptor -α
SERMs	Selective estrogen receptor modulators
SOCS3	Suppressor of cytokine signaling-3
SOD	Superoxidae dismutase
SQM	Squamous metaplasia
SRB	Sulfarhodamine-B
STZ	Streptozotocin
T	Testosterone
TBST	Tris-buffered saline plus Tween-20
TLR4	Toll like receptor 4
TNF-α	Tumor necrosis factor -α
TRAMP	The transgenic adenocarcinoma of the mouse prostate
VLCFA	Very long chain fatty acids
X-ALD	X-linked adrenoleukodystrophy
XO	Xanthine oxidase

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Aim of The Work

Prostate cancer (PC) remains among the most frequently diagnosed solid tumors. It is considered a worldwide public health problem and is the first cause of death by cancer in men over 50 years of age (Jemal et al., 2010).Treatment options for castration-resistant PC (CRPC) are limited and are often associated with significant morbidity and mortality. The high incidence of recurrence and metastasis, as well as the refractory nature of CRPC to chemotherapy, make it one of the most challenging malignancies for therapeutic drug combination studies (Diaz *et al*., 2004; Pinto *et al*., 2009). Taxanes were emerged as a powerful class of chemotherapeutic agents that proved therapeutic efficacy in various types of solid tumors including breast and PC. Paclitaxel (PTX) is a natural product isolated from the Pacific yew (Taxus brevifolia). Docetaxel (DOC) is a semisynthetic taxane produced from the European yew (Taxis baccata). In PC, DOC has demonstrated a survival advantage and is set to become a frontline therapy in the management of castration-resistant PC (Mackler *et al*., 2005; Magi-Galluzzi *et al*., 2007). However, the use of high dose of taxanes is often associated with significant toxic reactions (Fossella *et al*., 2000; Sewak *et al*., 2010). Since low or moderate doses of taxanes have no significant antitumor activity in patients (Ryan *et al*., 2002; Smith *et al*., 2011), it is crucial to investigate ways to reduce their dose without affecting the antitumor activity. Accordingly, natural products with anti-cancer efficacy and least toxicity to normal tissues are suggested as possible candidates to be investigated for their synergistic efficacy in combination with the conventional chemotherapeutic agents (Agarwal 2000).

Caffeic acid phenethyl ester (CAPE) is a phenolic component of honey bee propolis that possesses a plethora of biological activities. Several studies reported its broad-spectrum anticancer activity *in vitro* and *in vivo* in multiple cancer models, as colon cancer (Xiang *et al*., 2006), lung cancer (Chen *et al*., 2004),