BIOLOGICAL EVALUATION OF ANTI-ANDROGENIC EFFECT OF SOME PLANTS

By

KAREM EL-SAYED ALY FOUDA

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003
M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., 2010

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Biochemistry)

Department of Biochemistry
Faculty of Agriculture
Cairo University
EGYPT

2015

APPROVAL SHEET

BIOLOGICAL EVALUATION OF ANTI-ANDROGENIC EFFECT OF SOME PLANTS

Ph.D. Thesis
In
Agric. Sci. (Biochemistry)

By

KAREM EL-SAYED ALY FOUDA

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003
M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., 2010

Approval Committee

Dr. AHMED ALI ABDEL-RAHMAN .....
Professor of Biochemistry, Fac. Agric., Benha University

Dr. EMAM ABDEL-MOBDY ABDEL-REHEEM .....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. MAGDY ABDEL-ALEEM SHALLAN .....
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. MOHAMED MAGDY RASHED .....
Professor of Biochemistry, Fac. Agric., Cairo University

Date: 28/4/2015

SUPERVISION SHEET

BIOLOGICAL EVALUATION OF ANTI-ANDROGENIC EFFECT OF SOME PLANTS

Ph.D. Thesis
In
Agric. Sci. (Biochemistry)

By

KAREM EL-SAYED ALY FOUDA

B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Cairo Univ., 2003
M.Sc. Agric. Sci. (Biochemistry), Fac. Agric., Cairo Univ., 2010

SUPERVISION COMMITTEE

Dr. MOHAMED MAGDY RASHED
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. MAGDY ABDEL-ALEEM SHALLAN
Professor of Biochemistry, Fac. Agric., Cairo University

Dr. LAILA HANNA MOSAAD
Researcher Professor of Biochemistry and Nutrition, NRC, Giza

Name of Candidate: Karem El-Sayed Aly Fouda **Degree:** Ph.D.
Title of Thesis: Biological Evaluation of Anti-androgenic Effect of Some plants.
Supervisors: Dr. Mohamed Magdy Rashed
Dr. Magdy Abdel-Aleem Shallan
Dr. Laila Hanna Mosaad.
Department: Biochemistry **Approval: 28/4/2015**

ABSTRACT

The present study was designed to evaluate the anti-androgenic effect of some plants in castrated rats (flaxseed, sesame seeds, safflower seeds and soybean seeds) which were analyzed then petroleum ether and ethanol extracts were prepared from these samples. Some phytochemicals such as total phenolic compounds content, fatty acids, hydrocarbons and phytosterols contents were determined in all the studied plants. Also the anti-cancer effect of all the plants powder under investigation were studied against prostate cancer using cell-line technique. In the first experiment of the present study flax, sesame, safflower and soybean seeds were evaluated as anti-androgenic agents in castrated/testosterone rats in form of petroleum ether, ethanol extracts and whole powder. The nutritional safety of these studied plants and their extracts were evaluated through kidneys and liver functions. In the second experiment of this study, the most efficient treatments of the first experiment were studied in animal model of benign prostate hyperplasia (BPH) to evaluate the anti-androgenic effect of the plants under study in an animal model of BPH. Total cholesterol, malondialdehyde, testosterone, prostate specific antigen, acid phosphatase and levels of creatinine and urea were determined as indicator of kidneys function, while the activity of aspartate transaminase and alanine transaminase were determined as indicator of liver function. Whole plants powder showed anti-cancer activity against prostate carcinoma (PC3). All the studied agents in the first experiment showed significant anti-androgenic effect with different degrees through reduction of prostate weight and plasma levels of testosterone. Also plasma activities of AST and ALT showed non-significant changes in all studied groups relative to control. These results revealed that there was a complete safety of the present studied plants. It can be concluded that ethanol extract and whole studied plants powder showed promising effect towards BPH in animal model.

Key words: Antiandrogen, benign prostatic hyperplasia, flaxseed, sesame seeds, safflower seeds, soybean seeds and castrated rats.

ACKNOWLEDGEMENT

I wish to express my sincere thanks, deepest gratitude and appreciation to ***Dr. Mohamed Magdy Rashed*** *Professor of Biochemistry and* ***Dr. Magdy Abdel Alem Shallan*** *Professor of Biochemistry, Faculty of Agriculture, Cairo University for supervision, continued assistance and their guidance through the course of study and revision the manuscript of this thesis.*

I wish to express my sincere thanks, deepest gratitude and appreciation to ***Dr. Laila Hanna Mosaad*** *Researcher Professor of Biochemistry and Nutrition,* ***Dr. Doha Abdo Mohamed*** *Researcher Professor, National Research Centre, for suggesting the subject, for the extremely good research facilities, constructive supervision and criticism throughout the course of the work and* ***Dr. Reham Shehab EL Nemr*** *Researcher, National Research Centre for help me in pathohistological testing. In addition, many thanks to* ***Dr. Ibrahem Hamed*** *Researcher Professor of Biochemistry and Nutrition, National Research Centre for help me all the time and support me to complete this thesis.*

Also, Grateful appreciation to all staff members of Nutrition Department, National Research Centre.

LIST OF ABBREVIATIONS

5-αR	5-α Reductase
ALA	α-Linolenic Acid
ALT	Alanine Transaminase
AR	Androgen Receptor
ARE	Androgen Response Elements
AST	Aspartate Transaminase
BOO	Bladder Outlet Obstruction
BPH	Benign Prostate Hyperplasia
COX	Cyclo-oxygenase
CT	Castrated/Testosterone rats
CZ	Central Zone
DHT	Dihydrotestosterone
DMSO	Dimethyl Sulfoxide
E2	Estradiol
EFG	Epidermal Growth Factor
END	Entrodiol
ENL	Entrolctone
GPR	G-protein Coupled Receptor
HMR	7-hydroxymatairesinol
HSPs	Heat Shock Protein
IFN	Interferon
IGF	Insulin Growth Factor
IGFBP	Insulin Like Growth Factor Binding Protein
IL	Interleukin

KGF	Keratinocyte Growth Factor
LDL	Low Density Lipoprotein
LPS	Lipopolysaccharide
LUTS	Lower Urinary Tract Symptoms
MAT	Matairesinol
MDA	Malondialdehyde
MetS	Metabolic Syndrome
NSAIDs	Non-steroidal Anti-inflammatory Drugs
PAP	Prostate Specific Acid Phosphatase
PC	Prostate Cancer
PSA	Prostate Specific Antigen
PUFA	Polyunsaturated Fatty Acids
PZ	Peripheral Zone
SDG	Secoisolariciresinol Diglycoside
SECO	Secoisolariciresinol
SHBG	Sexual Hormone Binding Globulin
SM	Smooth Muscle
T	Testosterone
TBA	Thiobarbituric Acid
TNF	Tumor Necrosis Factor
TP	Testosterone Propionate
TPC	Total Phenolic Compounds
TURP	Transurethral Resection of the Prostate
TZ	Transition Zone

CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Testosterone (T), the most abundant androgen in serum, is synthesized and secreted by the testes (95%) and adrenal glands (5%). However, testosterone is not the primary androgen responsible for the development, growth and pathogenesis of the prostate (Roehrborn and Mcconnell, 2007 and Klein *et al*., 2007). Testosterone is converted by 5-α reductase (5-αR) to dihydrotestosterone, which is the more potent ligand for androgen receptor, and ligand binding to androgen receptor leads to an interaction with the androgen response elements of gene promoters (Hamilton and Freedland, 2011). Dihydrotestosterone is the most prevalent and potent form of androgen in various human organs and tissues and plays a crucial role in the pathogenesis and progression of several diseases such as benign prostate hyperplasia, prostate cancer, male pattern baldness, hirsutism and acne (Cilotti *et al*., 2001).

Anti-androgens are an assorted group of drugs and compounds that reduce the levels or activity of androgen hormones within the human body. An androgen antagonist (anti-androgen) also can broadly be defined as any compound that has the biological effect of blocking or suppressing the action of male sex hormones such as testosterone within the human body. Disease states in which this is relevant include polycystic ovarian syndrome, hirsutism, acne, benign prostate hyperplasia, and endocrine related cancers such as carcinoma of the prostate (Grant and Ramasamy, 2012). Androgen-related diseases impair the well-being of many aging men. Unfortunately, the medications used to treat these diseases have many side effects. Therefore, there is a significant need for the development of novel

drugs to treat androgen-related diseases (Chiu *et al.*, 2013). The presence of anti-androgenic phytochemicals in plants, herbs, and food stuffs provides an alternative to modern synthetic pharmaceuticals. It is also commonly believed that there are fewer adverse effects of such alternative therapies (Grant and Ramasamy, 2012). Anti-androgens can exhibit their activity both on a peripheral and a central site. They compete with the peripheral androgen receptors and thus inhibit the effect of endogenous or exogenous androgens. Inhibition of 5α-reductase is considered to be a mechanism to inhibit BPH (Akamine *et al.*, 2009). Phytoestrogens are defined as plant-derived compounds with estrogen like activities according to their chemical structures and activities (Jin *et al.*, 2013 and Roca *et al.*, 2014). Because of the similar molecular structures between phytoestrogens and endogenous steroid hormones, these phytoestrogens may have similar functions in our body (Kim and Choi, 2013). Exposure to low doses of phytoestrogens in the perinatal period affects Leydig cell function in adult rats, causing a decrease in testicular testosterone secretion (Akingbemi *et al.*, 2007).

Benign prostate hyperplasia (BPH) is one of the most common symptoms seen in older men, and 40% of men 50–60 years of age and 90% of those 80–90 years of age are diagnosed with benign prostate hyperplasia which has now become an atypical direct cause of mortality (Parsons, 2010, Nicholson and Ricke, 2011 and Zhang *et al.*, 2012). Logically, any treatment that could decrease androgen hormone action would have great potential in addressing prostate disorders, such as BPH or prostate cancer. There are already several medical treatments that act as androgen antagonists and have recognized uses,

however, in recent years, there has been an increasing demand for complementary and alternative therapies, and this has included an interest in the development and use of more plant-derived anti-androgen therapies. This is especially relevant as some medications currently in use have been found to have sub-optimal efficacy in clinical practice, and many patients are keen to try ‘natural’ or ‘alternative’ approaches as opposed to synthetically derived compounds (Grant and Ramasamy, 2012).

Therefore, the aim of the present work was to achieve the following:

1. Selection of some plant foods expected to be rich in phytoestrogen.
2. Since the chemical composition of crops varies with crop cultivars, soil and climatic conditions of the area, it is important to study the chemical composition of the studied plants before using any of them in the study.
3. Preparation of petroleum ether and ethanol extracts of all the studied plants.
4. Determination of some phytochemicals such as total phenolic compounds content, fatty acids, hydrocarbons and phytosterols contents in all the studied plants.
5. Evaluation of the anti-androgenic effect of the studied plants in castrated rats.
6. From results of the experiment of anti-androgenic effect, the most efficient treatments were studied in animal model of benign prostate hyperplasia.