

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการวิจัยแห่งชาติ



E42127

**EFFECTS OF ASPARAGUS RACEMOSUS ROOT EXTRACT
ON BONE AND REPRODUCTIVE ORGANS
IN OVARECTOMIZED RATS**

SURATSAWADEE CHUAMAN

**A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science Degree in Anatomy
February 2012
Copyright 2012 by Naresuan University**

600256252

ห้องสมุดงานวิจัย สำนักงานคณะกรรมการการวิจัยแห่งชาติ



**EFFECTS OF ASPARAGUS RACEMOSUS ROOT EXTRACT
ON BONE AND REPRODUCTIVE ORGANS
IN OVARIECTOMIZED RATS**



SURATSAWADEE CHUAMAN

**A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Master of Science Degree in Anatomy
February 2012
Copyright 2012 by Naresuan University**

This thesis entitled “Effects of Asparagus racemosus root extract on bone and reproductive organs in ovariectomized rats” submitted by Suratsawadee Chuaman in partial fulfillment of the requirements for the Master of Science Degree in Anatomy is hereby approved.

K. Ingkaninan Chair
(Associate Professor Kornkanok Ingkaninan, Ph.D.)

H. Kruevaisayawan Committee
(Hathairat Kruevaisayawan, Ph.D.)

Patcharada Amatyakul Committee
(Patcharada Amatyakul, M.D.)

Onrawee Khongsombat Committee
(Assistance Professor Onrawee Khongsombat, Ph.D.)

W. Weerachatanukul Committee
(Associate Professor Wattana Weerachatanukul, Ph.D.)

Approved

K. Papatwibul
(Assistant Professor Kanungnit Papatwibul, Ph.D.)

Dean of the Graduate School

27 February 2012

ACKNOWLEDGEMENT

The success of this thesis can be attributed to the extensive support and assistance from all my advisors, family, and friends.

First and foremost, my utmost gratitude to Dr. Hathairat Kruevaisayawan, my major advisor for accepting me to do research, guiding me with her excellent prospects in order to perform the experiments, and encouraging me with her intensive supervision to fulfill writing up this thesis. Above all and the most needed, she provided me encourage and support in many ways. Furthermore, I want to express my gratitude to her for using the precious time to read this thesis and gave me critical comments and helpful suggestions.

I would like to take this opportunity to express my gratitude to my co – advisors, Patcharada Amatyakul, MD. and Assoc. Prof. Dr. Onrawee Khongsombat who gave me valuable guidance in science knowledge, supervision, and support to complete this thesis.

I gratefully acknowledge to Assoc. Prof. Dr. Kornkanok Ingkaninan, faculty of Pharmaceutical Sciences, Naresuan University for kindly provide the *Asparagus racemosus* root extract for this study.

I wish to extend my warm thanks to my friends for help. Additionally, I gratefully thank all staff in faculty of Medical Science for their valuable suggestion and help throughout the study.

I would like to thank those whose names are not mentioned here but have greatly inspired and encouraged us until this thesis comes to a complete end.

Finally, I owe my loving thanks to my father and my mother for their unconditional love, special care, understanding, supporting throughout my life, encouragement which mean very much to me, and mental support which motivating my study to this success.

Suratsawadee Chuaman

Title EFFECTS OF ASPARAGUS RACEMOSUS ROOT
EXTRACT ON BONE AND REPRODUCTIVE ORGANS
IN OVARIECTOMIZED RATS

Author Suratsawadee Chuaman

Advisor Hathairat Kruevaisayawan, Ph.D.

Co – Advisor Patcharada Amatyakul, M.D.
Associate Professor Onrawee Khongsombat, Ph.D.

Academic Paper Thesis M.S. in Anatomy, Naresuan University, 2011

Keywords *Asparagus racemosus*, bone, osteoporosis, ovariectomy,
reproductive organs

ABSTRACT

E 42127

Osteoporosis is a bone disease caused mainly by estrogen depletion after menopause. Supplements of hormones have been developed to treat osteoporosis but they are associated with undesired adverse effects. Therefore, many natural estrogens have been considered as an alternative treatment. *Asparagus racemosus* (AR) is well known for its phytoestrogenic properties in stimulating female rejuvenation. This study aimed to evaluate the protective effect of the AR root extract on bone loss in ovariectomized (OVX) rat. Adult female Wistar rats were divided into five groups; 1) sham operated control (SHAM), 2) OVX control, 3) OVX+AR100, 4) OVX+AR1000 and 5) OVX+EE. OVX resulted in bone loss as evident by decreased femoral length, thickness and weight. In AR treated rats, these femoral parameters were enhanced significantly. OVX rats treated with AR showed an osteopreventing effect as the levels of the bone turnover markers, such as β -crosslaps (β -CTx), N-terminal propeptides of type I procollagen (P₁NP) and total alkaline phosphatase (ALP) were lower than OVX control rats. Histological examination of the bone in OVX rats revealed the loss of trabeculae and widening of intertrabecular spaces. AR root extract reversed these histological changes. The histomorphometrical results also confirmed the preventing effect of AR root extract. Furthermore, all dose treatment groups of AR root extract did not exert stimulatory effect on the uterus and mammary gland. The present study

E'42127

suggested that AR root extract may be effective for prevention of bone loss in rats induced by ovariectomy without causing undesirable effects in reproductive organs.

LIST OF CONTENTS

Chapter	Page
I INTRODUCTION	1
Rationale for the study.....	1
Main objective of the study.....	3
Specific objectives of the study.....	3
The scope of the study.....	4
Hypothesis.....	4
Research place.....	5
Research plans.....	5
II REVIEW OF RELATED LITERATURE AND RESEARCH	6
<i>Asparagus racemosus</i> Willd.....	6
Pharmacological properties of AR root	8
Phytoestrogens	15
Menopause	16
Bone loss	18
Plants, medicinal herbs and herbal supplements of osteoporosis.....	24
Uterus	28
Mammary gland	30
Animal model of osteoporosis	32
III RESEARCH METHODOLOGY	33
Instruments and materials.....	33
Methods	34

LIST OF CONTENTS (CONT.)

Chapter	Page
IV RESULTS	41
Effect of AR root extract on femoral parameters	41
Effect of AR root extract on biochemical parameters	43
Effect of AR root extract on calcium, phosphorus, ALP and estradiol level	45
Histological analysis of femur	48
Histomorphometric analysis of trabecular bone	51
Histological analysis of cortical bone	54
Histomorphometric analysis of cortical bone thickness	57
Body weight	58
Uterine weight	59
Histological analysis of uterus	61
Histomorphometric analysis of uterus	65
Histological analysis of mammary gland	66
Histomorphometric analysis of mammary gland	69
V DISCUSSIONS	71
Effect of AR root extract on femoral parameters	71
Effect of AR root extract on bone markers	72
Effect of AR root extract on calcium, phosphorus, ALP and estradiol level	73
Effect of AR root extract on femoral histology and histomorphometry	74
Effect of AR root extract on body weight	75
Effect of AR root extract on reproductive organs	76
VI CONCLUSIONS	77

LIST OF CONTENTS (CONT.)

Chapter	Page
REFERENCES	78
APPENDIX	94
BIOGRAPHY	97

LIST OF TABLES

Table	Page
1 Research Plans.....	5
2 Conclusion of the experiments evaluated on the pharmacological actions of AR	12
3 Symptoms of estrogen deficiency.....	18
4 WHO classification for diagnosis of osteoporosis using BMD measurement.....	22
5 Markers of bone formation and resorption.....	23
6 Effect of AR root extract on femoral parameters after 90 days of treatment.....	42
7 Effects of AR root extract on biochemical parameters after 90 days of treatment	47
8 Effects of AR root extract on rats body weight after 90 days of treatment.....	59
9 Effects of AR root extract on rats uterine weight after 90 days of treatment.....	60
10 Effect of AR root extract on thickness of endometrium and myometrium after 90 days of treatment	65

LIST OF FIGURES

Figures		Page
1	The characteristic of <i>Asparagus racemosus</i> Willd	6
2	The main active compounds of <i>Asparagus racemosus</i> Willd (I)Shatavarin, (II)Sarsasapogenin.....	7
3	Menopausal stages	17
4	Diagram of a long bone	19
5	Histological section (stained with H&E) of the long bone at diaphysis in longitudinal plane of rat showing two types of bone tissue: compact and cancellous bone. Scale bars = 200 μm	20
6	Turnover process.....	21
7	The characteristic of <i>Epimedium sagittatum</i> (left figure) and the chemical structure of icariin (right figure)	24
8	Terpenoid and sterol chemical structure	25
9	Daidzin and Genistin chemical structure.....	26
10	The chemical structure of puerarin	27
11	Anatomy of human uterus.....	29
12	Anatomy of rat uterus.....	29
13	Histological section (stained with Hematoxylin & Eosin) of the uterus of rat in transverse plane. Scale bars = 50 μm	30
14	Breeding female rats with an average of 12 mammary glands.....	31
15	Histological features of H&E-stained paraffin section of the mammary glands of female Wistar rat showing the epithelium, duct, adipose tissue and connective tissue.....	31
16	Experimental schedule	36

LIST OF FIGURES (CONT.)

Figures	Page	
17	Effect of AR root extract treatment on serum β -CTx measured in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bar represent mean \pm S.E.M. of six animals. ^a $p < 0.05$ and ^c $p < 0.001$ compared to SHAM; ^{##} $p < 0.01$ and ^{###} $p < 0.001$ compared to OVX.....	44
18	Effect of AR root extract treatment on serum P ₁ NP measured in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bar represent mean \pm S.E.M. of six animals. Bar represent mean \pm S.E.M. of six animals. ^b $p < 0.01$ compared to SHAM; ^{##} $p < 0.01$ compared to OVX.....	45
19	Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was SHAM operated control and orally administered with vehicle for 90 days.....	48
20	Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy.....	49
21	Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.....	49

LIST OF FIGURES (CONT.)

Figures		Page
22	Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.....	50
23	Histology of the distal femur at metaphyseal region in longitudinal plane in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy.....	50
24	Effect of AR root extract on the thickness of trabecular bone in various groups of rats. Treatments represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals. ^c <i>p</i> < 0.001 compared to SHAM; ^{###} <i>p</i> < 0.001 compared to OVX.....	52
25	Effect of AR root extract on intertrabecular space in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals. ^a <i>p</i> < 0.05, ^b <i>p</i> < 0.01 and ^c <i>p</i> < 0.001 compared to SHAM; [#] <i>p</i> < 0.05 and ^{##} <i>p</i> < 0.01 compared to OVX.....	53
26	Effect of AR root extract on trabecular area in metaphysis of distal femur in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals. ^b <i>p</i> < 0.01 and ^c <i>p</i> < 0.001 compared to SHAM; ^{##} <i>p</i> < 0.01 and ^{###} <i>p</i> < 0.001 compared to OVX.....	54

LIST OF FIGURES (CONT.)

Figures		Page
27	Cortical bone morphology in longitudinal plane of femur in animal that was SHAM operated control and orally administered with vehicle for 90 days.....	55
28	Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy.....	55
29	Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.....	56
30	Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy.....	56
31	Cortical bone morphology in longitudinal plane of femur in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy.....	57
32	Effect of AR root extract on cortical bone thickness in various groups of rats. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals.....	58
33	Uterine morphology in transverse plane in animal that was SHAM operated control and orally administered with vehicle for 90 days. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).....	62

LIST OF FIGURES (CONT.)

Figures		Page
34	Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).....	63
35	Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).....	63
36	Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).....	64
37	Uterine morphology in transverse plane in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy. The uterine compartments are epithelium (Ep), endometrium (E), myometrium (M) and uterine gland (G).....	64
38	Mammary gland morphology in animal that was SHAM operated control and orally administered with vehicle for 90 days showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).....	67

LIST OF FIGURES (CONT.)

Figures		Page
39	Mammary gland morphology in animal that was ovariectomized and orally administered with vehicle for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).....	67
40	Mammary gland morphology in animal that was ovariectomized and orally administered with 100 mg/kg B.W./day of AR root extract for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).....	68
41	Mammary gland morphology in animal that was ovariectomized and orally administered with 1000 mg/kg B.W./day of AR root extract for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A).....	68
42	Mammary gland morphology in animal that was ovariectomized and orally administered with 0.1 mg/kg B.W./day of EE for 90 days after ovariectomy showing the features of duct (D), fibrocollagenous tissue (F) and soft adipose tissue (A). Note the minute amount of secretion (S) in the lumen of duct resulting from stimulation of estrogen treatment.....	69
43	Effect of AR root extract on the glandular area of mammary gland. Treatment groups represented are SHAM, OVX, OVX+AR100, OVX+AR1000 and OVX+EE. Bars represent mean \pm S.E.M. of six animals. ^c $p < 0.001$ compared to SHAM; ^{###} $p < 0.001$ compared to OVX.....	70

ABBREVIATIONS

μm	=	micrometer
μm^2	=	squaremicrometers
$^{\circ}\text{C}$	=	Degree Celcius
A	=	adipose tissue
ALP	=	alkaline phosphatase
AR	=	<i>Asparagus racemosus</i>
β -CTx	=	beta-crosslaps
BMD	=	Bone mineral density
B.W.	=	Body weight
Ca	=	Calcium
cm	=	centrimetre
dL	=	deciliter
D	=	duct
DMBA	=	7,12-dimethylbenz[α]anthracene
D.W.	=	Distilled water
E	=	endometrium
EDTA	=	Ethylenediaminetetraacetic acid disodium salt dehydrate
EE	=	17 α -ethynylestradiol
Ep	=	epithelium
ER	=	Estrogen receptor
F	=	Fibrocollagenous tissue
g	=	gram
G	=	uteine gland
h	=	hour
H&E	=	Hematoxylin and eosin
HRT	=	Hormone replacement therapy
mg	=	milligram
min	=	minute
ml	=	milliliter

ABBREVIATIONS (CONT.)

mm ²	=	squaremeters
NaOH	=	Sodium hydroxide
ng	=	nanogram
Ostex	=	Aminoterminal cross-linking telopeptide of bone collagen
OVX	=	Ovariectomy
PG	=	Propylene glycol
pg	=	picogram
Pi	=	Inorganic phosphate
P ₁ NP	=	Procollagen type 1 amino-terminal propeptide
SERM	=	Selective estrogen receptor modulator
SHAM	=	Sham operate control
S.E.M.	=	Standard error of the mean
TRAP	=	Tartrate-resistance acid phosphatase
U	=	ungstrom