Effect of Isoflavones on Vagina of Senile Albino Rats: A Histological Study

Thesis

Submitted for partial fulfillment of master degree in Anatomy

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2018

سورة البقرة الآية: ٣٢

Acknowledgments

First and forever, thanks to ***Allah****, Almighty for giving me the strength and faith to complete my thesis and for everything else.*

I would like to express my deepest gratitude and appreciation to ***Prof. Dr. Hemmat Abdelkader Abdelhamid,*** *Professor of Anatomy and Embryology, Faculty of Medicine – Ain Shams University, for her generous support and guidance to help me to put this work in its best form and for being an ideal model of a professor to follow. It was indeed an honor to work under her supervision.*

It is my pleasure to express my unlimited gratitude and deepest thanks to ***Prof. Dr. Rania Ahmed Salah El-Din,*** *Professor of Anatomy and Embryology, Faculty of Medicine – Ain Shams University, for her kind assistance, faithful supervision, precious help, valuable advice and guidance she offer me to complete this study. No words of gratitude can equal her help and support.*

I would like also to thank ***Dr. Enas Anwar Bekheet,*** *Lecturer of Anatomy and Embryology, Faculty of Medicine – Ain Shams University, for the efforts and time she has devoted to accomplish this work.*

I feel greatly indebted to all my family without their great effort, encouragement, help and support this work could not become.

Nesma Abdelhady Hassan Ibrahim

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List of Abbreviations

Abbrev.	Full-term
BMD	: Bone mineral density
CARE	: Committee of Animal Research Ethics
ER α	: Estrogen receptor subtype alpha
ER β	: Estrogen receptor subtype beta
ERT	: Estrogen replacement therapy
GSM	: Genitourinary syndrome
HRT	: Hormone replacement therapy
LH	: Luteinizing hormone
LP	: Lamina propria (LP)
NK	: Natural killer cells
8-PN	: 8-prenylnaringenin
SHBG	: sex hormone binding globulin
SPSS	: Statistical Package for Social Science
VTE	: Venous thromboembolism
WHI	: Women's Health Initiative

Abstract

Introduction: The vagina is the copulatory organ in women. It's a distensible fibromuscular tube that extends from the perineum through the pelvic floor and into the pelvic cavity. Atrophy of the vaginal mucosa at menopause is accompanied by reduced fluid secretion, reduced levels of lactobacilli, and increased vaginal pH, and that these epithelial changes are responsible for the vulvovaginal symptoms or the genitourinary syndrome and its symptoms. Soy is considered a category of botanical supplement. Due to the ability of its major isoflavones to bind estrogen receptors, it has often been considered a likely alternative to estrogen replacement therapy in postmenopausal women

Objective: The objective of this study was to detect effect of isoflavones on structure of the vagina of senile rats using different histological techniques and to detect effect of isoflavones on thickness of the vaginal epithelium and the percent area of collagen in the lamina propria of the vagina using image analysis.

Methods: An experimental study was conducted on 24 female albino rats, 16 of them were aged 24 months, while 8 of them were about 6 months rats were divided into 3 equal groups of 8 animals: groupI (GI) received vehicle and represented adult control, groupII (GII) represented senile group and received vehicle, groupIII (GIII) received piscladene capsule. The one capsule contained 300 mg of the drug. Capsules were divided in a way to be administered by gavage in a dose 100mg/kg BW every day for 14 days. After that, the animals were killed under anaesthesia and the vagina was removed for histological and immunohistochemical analysis. Data were statistically analyzed using the SPSS software (statistical package for social studies-version13.0). One way analysis of variance (ANOVA) was employed to compare means in different groups with each other.

Results: GII showed signs of vaginal atrophy with decrease in the thickness of the epithelium, the area percent of collagen fibers, glycogen content and the decrease in the estrogen receptors subtype α. GIII presented increase in the thickness of the vaginal epithelium, collagen amount, glycogen content and promotion of estrogen receptors subtype α similar to that of the control group (GI)

Conclusion: The data of the present study suggest that atrophic changes occur in the rat vagina with aging, and that use of isoflavones improved these atrophic changes

Key words:
Isoflavone- vagina- atrophic changes

Introduction

The pathophysiologic changes associated with urogenital atrophy are a direct consequence of the decreased estrogen during the transition from peri-to postmenopause. The commonly recognized symptoms of this condition are vaginal dryness, itching, burning dyspareunia, and urinary incontinence **(Manonai et al., 2006).**

Clinical trials reporting increased risk of cardiovascular disease and breast cancer among women randomized to Hormone replacement therapy (HRT) have increased interest to other therapies of menopausal women **(Tice et al., 2003).**

Thus, the shift to alternatives and natural strategies was necessary for prevention and treatment of menopausal conditions. In that respect, soy products are of particular interest because of their potential health benefits in a range of hormonal conditions, probably due to their high content of phytoestrogens **(Mathey et al., 2007).**

Phytoestrogens are naturally occurring phytochemicals found in plants and plant products, which are structurally and functionally similar to 17β-estradiol (isoflavones). The principle phytoestrogens are the isoflavones (for example, coumestrol, genistein, daidzein, and equol) and lignins (for example, enterolactone and enterodiol), derived from

precursors in the diet by the gut microflora. Coumestrol is the most potent of the phytoestrogens, but is some 100–200 times less potent than 17β-estradiol, and almost 3000 times less potent than diethylstilbestrol. Phytoestrogens are present as glycosides in the diet in legumes, grains, nuts, and other fiber rich foods, and are present in the plasma and urine of both humans and animals eating a diet rich in such foods. They have trophic changes on female genital tract including uterus and vagina **(Burton and Wells, 2002).**

Various physiologic effects of soy food consumption have been attributed to the estrogenic actions of isoflavones **(Liwei et al., 2006).**

Improvement of vaginal dryness has been seen in many studies of soy, mixed soy isoflavones, or genistein treatment **(Crisafulli et al., 2004, Danna et al., 2007, Rosa Lima et al., 2014, Ghazanfarpour et al., 2015).**

So, soy rich diet should be considered in the preventive interventions against menopausal effects and vaginal atrophy (**Rosa Lima et al., 2017).** However**, (Mannonai et al., 2006)** reported that soy rich diet didn't relieve the urogenital symptoms, restore the vaginal epithelium or improve the vaginal health in peri- and postmenopausal women.

Aim of the Work

The objective of this work was to detect effect of isoflavones on structure of the vagina of senile rats using different histological techniques and to detect effect of isoflavones on thickness of the vaginal epithelium and the percent area of collagen in the lamina propria of the vagina using image analysis.

Normal Structure of the Vagina

The vagina is the copulatory organ in women. It's a distensible fibromuscular tube that extends from the perineum through the pelvic floor and into the pelvic cavity. Its internal end is enlarged to form what's called vaginal vault. The vagina also acts as a birth canal **(Speroff and Fritz**; **2011).**

The vagina is oblique downward and forward; that's why its anterior wall is 7.5 cm and its posterior wall is 9 cm. the vaginal wall consists of three layers: Mucosa, musculosa and adventitia. The mucosa is formed of stratified squamous epithelium with an underlying lamina propria. The epithelium consists of basal/pre basal layer, mid zone and a superficial layer. The lamina propria consists of a loose fibrovascular stroma containing elastic fibers and nerves. The muscular layer consists of inner circular layer and outer longitudinal layer of smooth muscles. The adventitia contains numerous blood vessels and nerves within adipose tissue **(Buy and Ghossain, 2013).**

In the fertile period of a woman's life, the light microscopic studies indicate four main epithelial cell layers: the basal layer, the mitotic active supra- or parabasal layer, the intermediate glycogen-containing layer, and the non-cornified superficial layer with pyknotic nuclei. The vaginal mucosa harbors a varying number of lymphocytes, granulocytes, and

macrophages. Macrophages are stimulated by estrogens to perform phagocytic activity which could be a positive factor in resistance to infections. Lymphoid nodules occur with T and B cells. This invasion of leucocytes especially that of lymphocytes is cycle dependent with a peak at menstruation while the electron micrographs showed densely packed cells in the superficial layer with narrow, cycle-independent, inter-cellular spaces. Deeper in the epithelium the inter-cellular spaces widen and become large; the maximum development is attained during the ovulatory and luteal phases (**Frosberg 1995)**.

Gandhi et al. (2016) stated in their review that the vaginal wall is constantly exfoliating and producing glycogen which is hydrolyzed to glucose. The predominant lactobacillus of healthy vaginal flora metabolizes glucose into lactic and acetic acid lowering the vaginal pH to a range of 3.5- 4.5. This acidity of the vagina provides natural protection against urinary tract infection and vaginitis, discouraging the growth of pathogenic bacteria and infection. Estrogen is essential for modulating innate immunity of the urinary tract.

The epithelial surface is moistened by fluid, partly originating from the uterus, partly being a transudate from the sub-epithelial vascular bed and then passively transported through the epithelial intercellular spaces to the surface. In

non-keratinized epithelia, tight junctions act as Para cellular diffusion barriers **(Frosberg, 1995).**

The vagina is also lubricated by cervical mucus, which is derived from the rich vascular network, and during sexual arousal lubricating mucus is also provided by glands at the vaginal vestibule, including the paired greater vestibular glands of Bartholin **(Mescher et al., 2013)**.

Estrogen is a vasoactive hormone that increases blood flow which aids vaginal lubrication through fluid transudation from blood vessels**.** Estrogen also helps in formation of vaginal rugae as activated estrogen receptors encourage epithelial proliferation with redundant smooth muscle layer. This formation of rugae helps in expandability, distensibility, and vaginal lubrication of the vagina during sexual stimulation **(Gandhi et al., 2016).**

Anatomy of the vagina of the rat

Gross Appearance. The vagina is a distensible, thin-walled tubular structure which extends from the cervix to the vulva. It is loosely attached to the rectum dorsally and to the urethra ventrally. In the adult rat, the vagina measures about 15-20 mm in length and, when distended, the diameter measures 3-5 mm. It is flattened dorso-ventrally with numerous longitudinal folds which increase in width posteriorly and

continue to the periphery of the vaginal opening. The appearance of the vaginal mucosa varies, depending on the stage of the estrous cycle. It is dry, without luster, and opaque during proestrus and estrus, and moist and pinkish during metestrus and diestrus **(Long and Evans, 1922)**.

Histological Features: The vagina consists of an inner layer of mucosa, a middle layer of musculosa, and an outer layer of adventitia. The mucosa, which undergoes characteristic changes during the estrous cycle, is composed of stratified epithelium and the underlying lamina propria. The lamina propria is fibrous and consists of a considerable number of fibroblasts embedded in an interwoven collagenous meshwork **(Burack et al., 1941)**. The musculosa is composed of several layers of smooth muscle arranged longitudinally and obliquely at the anterior end of the vagina. It gradually decreases in thickness, with only longitudinal fibers remaining in the middle region of the vagina. At the posterior end, it is reduced even further and only a few fibers are found in this region. Also present in this region are a few irregularly arranged striated muscle fibers intermixed with the connective tissue. The adventitia, which encompasses the vessels and nerves, is composed of a thin layer of connective tissue that is continuous with the connective tissue surrounding the rectum and urethra **(Jones et al., 1987).**

Merkwitz et al. (2016) reported that vaginal epithelium of the mature female mice showed structural differences during the four stages of the estrous cycle. They stated that vaginas with small stratified non-cornified epithelium and plump polygonal proliferating cells were assigned to the diestrus stage. Those with a stratified non-cornified epithelium with an outer superficial layer of mucified cylindrical cells were referred to as the proestrus stage. Vaginas with a tall stratified squamous epithelium with a broad stratum corneum and a complete loss of mucified cells were assigned to the estrus stage. Finally, vaginas with a slight to massive transepithelial migration of leucocytes and a partial to complete loss of the cornified layers were assigned to the metestrus I and II stages, respectively**.**

Westwood (2008) stated that formation of the stratum granulosum over the stratum germinativum of the vaginal epithelium, consisting of flattened epithelial cells containing many keratohyalin granules, marks the start of proestrus. Following the early formation of the stratum granulosum, there is a progressive development of the superficial mucoid, characterized by layers of cuboidal to ovoid cells with mucin containing cytoplasmic vacuoles, and the formation of a stratum corneum of dense, cornified cells. There is little if any degeneration or desquamation during the early- or mid-proestrus period. At the end of the proestrus phase, the epithelium is fully cornified and generally shows a superficial mucoid layer

exhibiting some desquamation of mucoid cells, while in the estrous phase, there is a loss of mitotic figures and a progressive shedding of the superficial mucoid and cornified layers. The end of this stage is characterized by detachment of the cornified epithelium, although some may persist, particularly adjacent to the vaginal opening.

Estrogen receptors in the vagina

Carbonel et al. (2011) reported that there are two subtypes of rat, mouse, or human ERs were identified, namely, ER-α and ER-β, that have different tissue distributions and transcriptional regulatory effects on a wide number of target genes. ER-α is expressed most abundantly in the female reproductive tract (uterus, vagina, and ovaries) and mammary glands, whereas ER-β is expressed mostly in prostate gland and ovaries. As stated by **(Manonai et al., 2007)** ERs in vagina were expressed in the vaginal epithelial, stromal, and vascular smooth muscle cells.

In the rat vagina **(Wang et al., 2000)** stated that ERα mRNA levels were increased in the stroma during metestrus. However, the epithelium exhibited a significant increase in ERα mRNA levels from estrus to metestrus. ERα mRNA was abundantly expressed in the epithelium and stroma during metestrus and diestrus, and in basal/parabasal cells during proestrus. They also showed that ERα is the predominant ER