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The Effect of 17β-Estradiol and Genistein on the Prostate Gland and Testes of Aged Rats

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Abstract

This experiment was carried out to investigate the effect of 17β-estradiol and genistein, a phytoestrogen, on the primary and the secondary male organs of aged male rats and related reproductive hormones. The effect of each chemical was evaluated using several tests. The effect of 17β-estradiol and genistein on the histological structures of the testes and the prostate gland was evaluated by Hematoxylin and Eosin staining. The ability of 17β-estradiol and genistein to affect the proliferation capacity of the prostatic cells was determined by the immunolocalization of the proliferating cell nuclear antigen (PCNA). Finally, the pituitary-gonadal hormonal interplay was evaluated by ELISA which was used to determine the changes that occurred in the blood hormone levels of testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH). The results showed that the 17β-estradiol significantly reduced the gross weights of the prostate and testes. This reduction of the weight was accompanied by a prominent degeneration of the secretory epithelial cells of the prostate and the seminiferous tubules of the testes. In addition, 17β-estradiol acts to inhibit the proliferation of the prostatic cells as evident by a significant reduction of the PCNA proliferation index. Although 17β-estradiol treatment was associated with significant decrease of blood testosterone level, it exerts no significant effect on either LH or FSH levels. On the other hand, genistein did not show any effect on the prostate, testes or blood hormone levels when compared to the control group. These results indicated that 17β-estradiol exerts a deleterious effect on the structure of the prostate, testes, and testosterone production without any appreciable effect on the pituitary hormones.

Keywords: Prostate, Testes, 17β-estradiol, Genistein, Testosterone, Proliferation Index

1. Introduction

Exposure of animals to a naturally occurring and synthetic estrogenic compounds, such as 17β-estradiol have been shown to affect male reproduction by interfering with the testicular structure and function (Gill-Sharma *et al*., 2001). These estrogenic compounds are also implicated in the disruption of normal endocrine functions of the hypothalamic-pituitary-gonadal axis, therefore posing a potentially serious male fertility problem (Gill-Sharma *et al*., 2001). Of particular concern is the effect of the estrogenic compounds on the testicular androgen production (Jones *et al*., 1978), which might influence the prostate gland growth, as its growth and maturity depend on the continuous supply of testosterone (Shidaifat *et al*., 2007). Indeed, it has been shown that estrogen treatment of neonatal rodents acts to decrease the number of estrogen receptors and alter prostatic cell proliferation (Un-No et al., 2007), and differentiation (Putz *et al*., 2001).

Benign Prostatic Hyperplasia (BPH) is a spontaneously occurring condition of aged males (Oesterling, 1996) with hormones being the major role players of such progression (Nicholson *et al.*, 2013; Nicholson and Ricke, 2011). Although androgens are the primary hormones that cause such a condition, estrogen also has its contribution by targeting the estrogen receptor alpha (ERα) and beta (ERβ) (Gallardo *et al*., 2009). While estrogenic effect mediated by ERα contributes to the pathogenesis of BPH (Shi *et al.*, 2017), estrogenic effects mediated by ERβ act to suppress prostatic cells proliferation, and support their differentiation (Christoforou *et al.*, 2014; Prins and Korach, 2008). Therefore, it is reasonable to assume that activation of ERβ with its antiproliferative feature has the potential to play a suppressive role, and thus could be used to control BPH.

Genistein, a non-steroidal chemical derived from soybeans, exerts an estrogen like activity on vertebrates' tissues. It has been reported that genistein consumption is associated with lower risk of many cancers, including the prostate cancer (Jaiswal *et al.*, 2019). Genistein appears to reduce the incidence of prostate adenocarcinoma through a mechanism that involves, at least, the down regulation of ERα (Lamartiniere *et al.*, 2002), and modulation of its activation (Kostelac *et al.*, 2003). Together, these findings suggest a suppressive role of genistein on the growth, and development of the prostate gland, as well as providing a potential therapeutic value to treat prostate gland modalities. This study was conducted to compare the effect of estradiol and genistein on the testes, the prostate gland, and the associated endocrinological interplay of the pituitary-gonadal axes in aged rats.

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2. Materials and Methods

2.1. Animals

Fifteen male Wistar rats, aged between 10 – 12 months were used in this study. They were housed in the Animal House at the Jordan University of Science and Technology. The rats were randomly assigned to one of three groups (n=5). The first group served as a control and rats of this group received a daily injection of 0.1 ml of dimethyl sulfoxide (DMSO) for 26 days. The rats of the second group were treated daily with Genistein (10mg/0.1ml) for 26 days. The rats of the third group received treatment of estrogen (1mg/0.1ml) for 26 days. At the end of the treatment period, all rats were euthanized in a jar that contained ether-soaked cotton. Samples were then collected from each rat and included the prostate gland, testes, and blood. All procedures of animal handling, and sample collection were approved by the Animal care and use committee of the Jordan University of Science and Technology.

2.2. Preparation of tissue and blood samples

Upon euthanasia of rats, the prostate glands, and the testes were collected and weighed. The prostate glands and testes were then fixed in a 4% buffered formaldehyde for 4 hours, after which the samples were processed and embedded in paraffin. The blood samples were centrifuged at 2500 rpm for 10 minutes, then serum was collected from each sample and stored for subsequent hormonal assay by Enzyme-linked Immunosorbent Assay (ELISA).

2.3. Hematoxylin & Eosin staining

Five µm thick sections were cut and mounted on microscopic glass slides. Sections were deparaffinized and hydrated and were then stained in Hematoxylin and Eosin.

2.4. PCNA Index

The proliferation rate of prostatic cells was determined as previously described (Shidaifat *et al.*, 2013). Briefly, nonspecific binding was blocked by with Power Block® (BioGenex, CA) and polyclonal antibody against PCNA (MyBioSource, San Diego, CA) was added. Sections were then covered with horseradish peroxidase, Mach 3 Rabbit HRP-Polymer® (BioCare, Concord, CA) in the humidified chamber and after washing the DAB (DakoCytomotion, Glostrupe Denmark) was added. Finally, tissues were counter stained with Mayer's Hematoxylin, and then visualized under light microscope. The PCNA index was determined by counting 500 epithelial cells in a random field from each sample, and then the percentage of positive cells among the 500 cells was calculated.

2.5. ELISA

Serum sample were used to measure the concentration of hormones according to the manufacture's instructions provided with the ELISA kit. Testosterone was measured using the kit supplied by BioCheck (Foster city, CA), whereas LH and FSH were measured using kits supplied by MyBioSource (San Diego, CA).

2.6. Statistical Analysis

Statistical analysis was performed using one-way analysis (ANOVA). Results were presented as the mean ±SEM. Differences were statistically significant at $p<0.05$.

3. Results

The results show that 17β-estradiol exerted a prominent effect on the testes and the prostate gland structures. Estradiol treatment induced a significant decrease in the weight of the testes (Figure 1), and its testosterone production (Figure 2) when compared to the control group. However, genistein treatment exerted no significant effect on the weight of the testes or its testosterone production.

The decrease in the weight of the testes, caused by estradiol treatment, was associated with prominent change in the histological structure. While the testes of a control (Figure 3A) and genistein treated rats (Figure 3B) appear to have a normally rounded circumference of seminiferous tubules containing germ cells at different stages of development, the seminiferous tubules of the estradiol treated rats appeared collapsed, shrunken, and contained disorganized cells (Figure 3C).

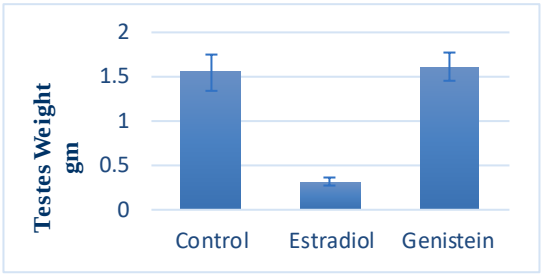

Figure 1. Comparison between the weights of testes of rats treated with estradiol and genistein for 26 days with the control group. Estradiol treatments caused a significantly ($P< 0.05$) decrease of testes weight as compared to control.

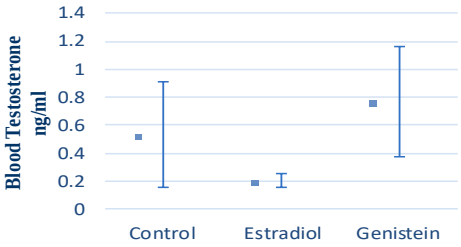

Figure 2. Concentration of serum testosterone of rats treated with estradiol and genistein for 26 days and the control group. Estradiol treatment causes a significantly ($P< 0.05$) decrease of testosterone as compared to control and genistein treated rats.

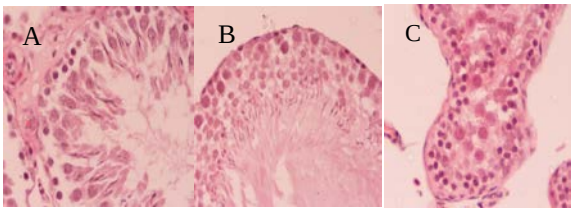

Figure 3: Testicular structure of rats treated with genistein and estradiol for 26 days and the control group . A) The seminiferous tubules of the control group appear normal with a rounded exterior along with the presence of cells developing from the basal layer towards the lumen. B) Rpresents the seminiferous tubules of testes from genistein treated rats, which appear simlier to that of the conrol. C) Represents the seminefious tubules of rats treated with estradiol. The semineferious tubules are collaped and contain disorganized cells.

Similarly, estradiol treatment caused a significant decrease in the weight of the prostate gland when compared to the control group (Figure 4). The histological evaluation revealed that the prostate gland from the estradiol-treated rats suffered prominent structural changes. While the acini of the prostate glands from the control (Figure 5A) and genistein treated rats (Figure 5B) appeared to contain a tall cuboidal secretory cell, the acini of the prostate gland from estradiol-treated rate appeared to contain atrophied cells with scant cytoplasm and prominent nuclei (Figure 5C).

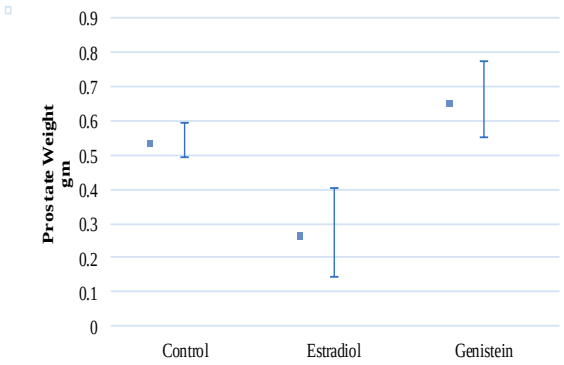

Figure 4. Comparison of weights of the prostate gland of rats treated with estradiol and genistein for 26 days and the control group. Estradiol causes a significant (P<0.05) decrease of prostate weight as compared to control.

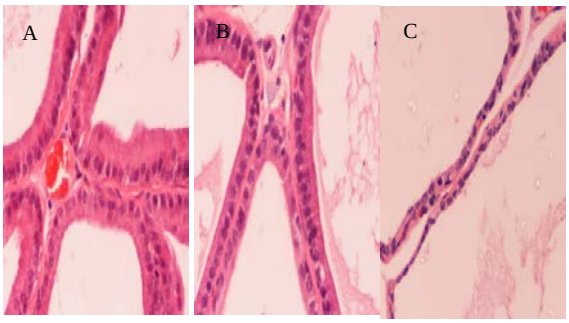

Figure 5. Comparison of the histological structures of the prostate treated for 26 days. (A) Prostate gland of control rat. Note that the glandular acini contain tall cuboidal epithelial cells. (B) Prostate gland from genistein-treated rats. The glandular acini retain similar structure to that of the control. (C) Prostate gland of estradiol treated rats. The acini contain atrophied cells with scant cytoplasm.

These structural changes of the prostate gland from estradiol-treated rats are paralleled with a significant decrease in the active proliferating cells. Estradiol treatment was associated with a significant reduction in the percent of cells that are expressing PCNA as evident by the PCNA proliferation index (Figure 6).

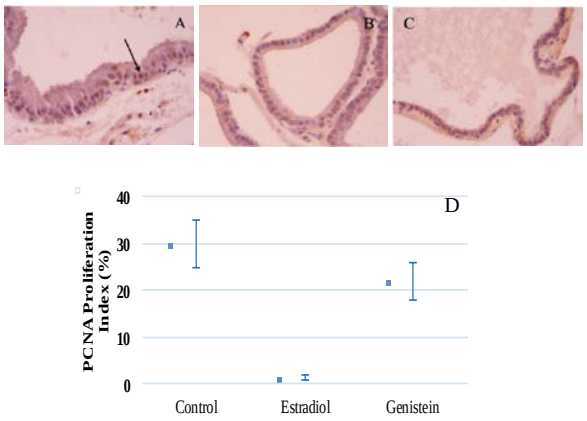

Figure 6. Expression of PCNA by the prostatic cells of rats treated for 26 days with genistein or estradiol. The arrow points to stained nuclei of actively proliferating cells. A) Control group, B) Genistein treated rats, and C) Estradiol treated rats. PCNA Proliferation index of rats treated with estradiol or genistein for 26 days (D). Estradiol treatment induced a significant (P<0.05) decrease in the percent of proliferating cells.

On the other hand, estradiol and genistein treatment exerted no significant effect on the serum level of FSH and LH (Figure 7 and 8 respectively).

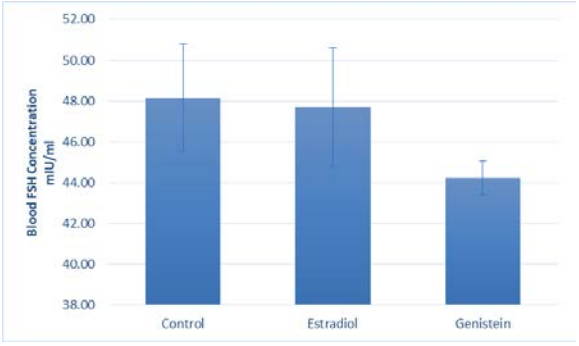

Figure 7. Blood concentration of FSH of rats treated with estradiol and genistein for 26 days as compared to the control group. There is no significant difference (P< 0.05).

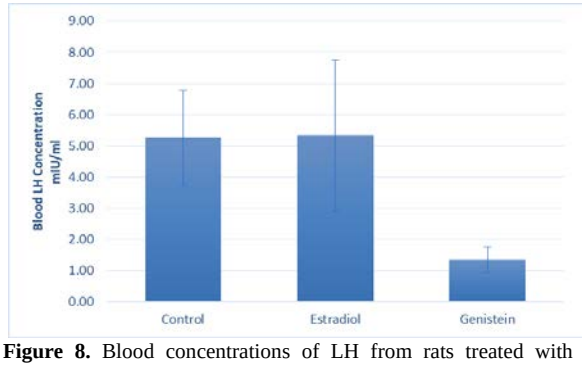

Figure 8. Blood concentrations of LH from rats treated with estradiol and genistein for 26 days compared with the control group. There is no significant difference (P< 0.05).

4. Discussion

The findings of this study indicate that estradiol treatment induced a significant decrease in the weight of the testes, which appeared to contain collapsed and shrunken seminiferous tubules with disorganized cells. The damage caused to the testicular structure by 17β-estradiol is like that induced by zeranol, a non-steroidal estrogenic derivative of the myco-estrogen zearalenone (Shidaifat *et al.,* 2013). In addition, it has been shown that elevated levels of estradiol alter the seminiferous tubules morphology (Gill-Sharma *et al.,* 2001) and impaired spermatogenesis (Leavy *et al.,* 2017), sperm count and motility (Mohammadzadeh *et al.,* 2021).

Interestingly, the effect estradiol on the testicular structure was accompanied by a significant reduction in testosterone production without any appreciable effect on FSH and LH levels. These results suggest a direct effect of estradiol on the testicular cells. In fact, it has been reported that estradiol exerts a direct effect on leydig cells steroidogenesis without interrupting the hypothalamus-pituitary-gonadal axis (Jones *et al.,* 1978).

The deleterious effect of estradiol was extended to the prostate gland, which appeared to be significantly smaller and suffered a prominent histological alteration of its glandular compartment. These structural changes of the prostate gland obtained from the estradiol-treated rats were accompanied with a significant reduction of the proliferation potential of the prostatic cells. Previously, we have demonstrated that testosterone is the only hormonal factor that is required to drive prostate gland development to maturity (Shidaifat *et al*., 2007), and its deprivation is associated with a dramatic regression of the gland (Shidaifat *et al.,* 2004). Therefore, the ability of estradiol to significantly decrease serum testosterone levels (as shown in this study), and to impair testicular steroidogenesis (Adibnia *et al.,* 2016) implicates a potential indirect effect of estradiol on the structural, and functional integrity of the prostate gland through its effect on the testicular androgen.

On the other hand, genistein as a naturally occurring substance related to the isoflavonoids with an estrogenic potency less than that of 17β-estradiol (Leffers *et al.*, 2001) has been shown to inhibit the growth of both BPH, and prostate cancer (Bektic *et al*., 2005; Geller *et al*., 1998). Despite the evidence shown for such activity of genistein, this study revealed that animals treated with genistein had unchanged levels of LH or FSH, hence, unchanged levels of testosterone. As a result, the gross mass, structure of prostate gland and the cellular proliferation showed no significant changes. Although there is evidence that males ingesting genistein as part of their diet are less exposed to BPH (Bektic *et al.,* 2005), aged rats used in this study were only exposed to genistein injections for 26 days, a period that may have been insufficient to induce any visible effect.

5. Conclusion

The results of this study supported and extended the existing evidence of the 17β-estradiol effects on the growth of the primary and the secondary sex organs. The effect of 17β-estradiol on the prostate gland appears to be indirect and probably mediated by disruption of the testicular androgen production. In contrast, genistein at a dosage and duration used in this study appears to exhibit no significant role on the structures of the male reproductive system, particularly the prostate gland. Although the results of this study cast doubt on the therapeutic potential of genistein for treating prostate gland modalities, further studies using different genistein treatment regimens and protocols are warranted.

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