The effect of phytoestrogen (lignan) on the levels of some hormonal parameters in white female rats induced osteoporosis

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Abstract

Background and Objectives: Osteoporosis is a widespread health problem affecting more than 75 million people worldwide. The objective of the current study was to evaluate the effectiveness of the phytoestrogen (lignan) in some hormonal parameters of white female rats induced osteoporosis.

Methods: The study included two main experiments, In the first experiment 50 adult white female rats were divided into two groups, The first group G1 was as a control group and the second group, G2 was induced into osteoporosis by Prednisolone at a dose of 40 mg/kg of B.W For two months. The second main experiment divided the remaining animals (from the first group of the first experiment into three subgroups) and the remaining animals (from the second group of the first experiment into two subgroups): negative control group C was dosed with distilled water, group T1 was as a positive control that was continued with Prednisolone at a dose of 40 mg/kg of B.W, group T2, was induced osteoporosis and administered the phytoestrogens(lignan) at a dose of 20 mg/kg, the healthy T3 group was administered the phytoestrogens(lignan) at a dose of 20 mg/kg, and the T4 group was administered the phytoestrogens(lignan) at a dose of 20 mg/kg. And Prednisolone at a dose of 40 mg/kg simultaneously for 30 days. After the end of the second experiment, the weights of the animals were recorded and then anesthetized
with chloroform withdrawal blood samples from the heart directly for hormonal tests that represent estrogen, FSH, LH.

**Results:** The results showed a significant decrease (P<0.05) in each of the average estrogen, FSH, LH level in the T1 group (positive control) when comparing to the negative group. while the cause of the phytoestrogen(lignan) improved The hormonal parameters and showed a significant increase (P<0.05) in estrogen, FSH, LH level in T2,T3,T4groups when comparing to T1 (positive control).

**Conclusion:** We conclude from the current study the effective and positive role shown by phytoestrogens(lignan) in improving and enhancing the hormonal parameters and reducing the negative effects of induced osteoporosis in white female rats.

**Keywords:** Phytoestrogen, osteoporosis, hormonal parameters, female rats.

1-Introduction

Osteoporosis (OP) is a serious health problem. About 200 million people in the world are at risk of this problem. Recent epidemiological studies have indicated that the incidence of osteoporosis varies between populations, as a result of the complex interaction between a variety of genes and factors Geographical and ethnic[1]. Osteoporosis occurs as a result of an imbalance between the function of osteoblasts and osteoclasts as a result of many factors, including natural and surgical menopause, as well as lack of exposure to ultraviolet radiation, low calcium and vitamin D in food, addiction to smoking, alcohol consumption, and glucocorticoids [2,3]. The pathophysiology of osteoporosis due to estrogen deficiency has been identified in both the early and late accelerated phases of decreased bone mass in postmenopausal women. The accelerated phase is evident during the first decade after menopause. function of bone cells[4,5]

Flaxseed (*Linum Usitatissimum*) is one of the oldest cultivated plants. Flaxseed contains the highest percentage of phytoestrogen content, which is also found in soybean products, legumes, fruits, vegetables, and
grains, as its content varies in different foods. Phytoestrogens are a variety of plant-derived compounds that have a structure similar to estrogen in mammals by containing a hydroxyl group and the phenolic ring and show many benefits to human health such as reducing the incidence of cardiovascular diseases, breast, and prostate cancer, and it also protects against menopausal diseases, including Osteoporosis as a result of the ability of phytoestrogens to stimulate the activity of osteoblasts and inhibit the formation of osteoclasts. Therefore, the current study aimed to study the effectiveness of phytoestrogens in flaxseeds in treating osteoporosis induced by prednisolone in female white rats.

2-Materials and methods
(2.1): Study Ethic approval
This study is approved and performed according to regulation of Ethical Committees/ College of Science at Al-Qadisiyah University.

(2.2): Preparation of phytoestrogen(lignan)
Pure phytoestrogens(lignan) were obtained from the Amazon website in the form of a powder, and the dose was prepared according to [7] concentration of 20 mg / kg / body weight.

(2.3): Experimental Animals
In this experiment, 50 adult female white rats whose age ranges between (3-4) months with weights ranging between 170-200 gm were used, obtained from the College of Science / University of Al-Qadisiyah. An average of 12 hours of light and 12 hours of darkness. The animals were left for a week for the purpose of adaptation. During the period of their adaptation, they were freely provided with water and diet.

(2.4): Design of Experiment
The first experiment
It included the process of inducing osteoporosis in female white rats, which were divided as follows:
The first group G1: included (26) healthy animals, and was considered a control group.

The second group G2: included (24) animals in which osteoporosis was induced by administering Prednisolone at a dose of 40 mg/kg of body weight according to [8] for two months. It was ascertained that osteoporosis by measuring the hormone estrogen, calcium, and vitamin D, and performing histological sections of the bone.

The second experiment

It included a study of the effect of the oral dose of phytoestrogen, as the remaining animals from the first group of the first experiment were divided into three subgroups and the remaining animals from the second group of the first experiment were divided into two subgroups as follows:

1- A negative control group C: included (7) animals who were dosed with normal saline only for a month.

2- The first treatment group T1: included (7) animals were continued to give Prednisolone at a dose of 40 mg/kg of body weight by (1) ml for a month and was considered positive control

3- The second treatment group T2: included (7) animals with developed osteoporosis, and phytoestrogens were dosed at a dose of 20 mg/kg of body weight by (1) ml for a month.

4- The third treatment group T3: included (7) healthy animals, phytoestrogens were dosed at a dose of 20 mg/kg of body weight, by (1) ml, for one month.

5- The fourth treatment group T4: included (7) healthy animals who were given phytoestrogen at a dose of 20 mg/kg and Prednisolone at a dose of 40 mg/kg of body weight by (1) ml simultaneously and for a month.

(2.5): Collecting blood samples

animals were anesthetized at the end of the second experiment, and blood was drawn directly from the heart, using a medical syringe with a capacity of 5 ml, and placed in tubes without anticoagulant, then placed in a centrifuge at a speed of 3000 rpm for 15 minutes, and then The serum
was kept in Eppendorf tubes at a temperature of -20 °C until it was used in measuring hormonal parameters.

(2.6): Measurement of hormonal parameters
Hormonal parameters (E2, FSH, LH,) were measured using the Cobas e 411 devices and kit from the German company Roche. This device is based on ElectroChemiLumenisce (ECL) technology.

(2.7): Statistical Analysis
The statistical program known as the statistical package for human sciences SPSS version 27 was used, where the analysis of unilateral and bilateral variance tests were applied with the calculation of the value of the least significant difference LSD for the purpose of comparing the averages of the treatments included in the study, and the T test was applied and calculated, and significant differences were identified at the level of probability 5 % [9].

3. Results
(3.1): Changes in the level of estrogen
The current results recorded in Table (1) revealed a significant decrease (P<0.05) in the level of estrogen hormone in treatment T1 compared with the negative control, while a significant decrease (P<0.05) was observed in the level of estrogen hormone in treatments T2 and T4 at Compared with negative control, T3 treatment showed a significant increase (P<0.05) compared with negative control.

The results shown in Table (1) revealed a significant increase (P<0.05) in the estrogen hormone in treatments T2, T3, and T4 compared with treatment T1, and treatment T3 showed a significant increase (P<0.05) in the estrogen hormone when comparing to T2, while treatment Regarding T4 treatment, it showed no significant differences (P>0.05) in the level of estrogen compared with T2, and a significant decrease (P<0.05) when comparing to T3.

(3.2): Changes in the level of follicle-stimulating hormone (FSH)
The results indicated in Table (1) that there was a significant decrease (P<0.05) in the level of FSH hormone in treatment T1 when comparing
to negative control, and it was also noted that there were no significant differences (P>0.05) in FSH in T2 and T3 when comparing to negative control, treatment T4 recorded a significant (P<0.05) decrease in FSH level compared with the negative control. The results recorded in Table (1) indicate that there is a significant increase (P<0.05) in FSH in treatments T2, T3, and T4 when comparing to treatment T1, and no significant differences (P>0.05) were observed between treatments T2 and T3, as recorded. The results of treatment T4, there were no significant differences (P>0.05) when comparing to T2, and a significant decrease (P<0.05) in FSH level when compared to T3.

(3.3): Changes in the level of the luteinizing hormone (LH)

In Table (1), the results recorded a significant decrease (P<0.05) in LH hormone in treatment T1 compared with the negative group, and a significant decrease (P<0.05) in LH in treatments T2 and T4 when comparing to the negative control, no significant differences were observed (P>0.05) between the treatments T3 and the negative control.

The results also indicated that there was a significant increase (P<0.05) in the level of LH in treatments T2, T3 and T4 compared with treatment T1, and a significant increase (P<0.05) in treatment T3 in LH compared with T2, with regard to treatment T4 there was a significant increase P (<0.05) in LH level when comparing to T2, while it showed a significant decrease (P<0.05) compared to T3.

Table (1): The effect of phytoestrogens (lignans) in flaxseeds on the level of some hormonal parameters in female white rats induced osteoporosis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
</tr>
</thead>
</table>

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Values are expressed as mean ± Standard Error (SE)

The vertically different letters indicate significant differences (P <0.05) between the study groups for each criterion.

The vertically similar letters indicate no significant differences (P> 0.05) between the study groups for each criterion.

C: Negative control group
T1: Positive control group.
T2: A group of induced osteoporosis rats was dosed with phytoestrogens at a dose of 20 mg/kg for 30 days.
T3: A group of healthy rats was dosed with phytoestrogens at a dose of 20 mg/kg of body weight for 30 days.
T4: A group of rats that were given phytoestrogens at a dose of 20 mg/kg and prednisolone at a dose of 40 mg/kg simultaneously for 30 days.

### 4. Discussion

(4.1) Hormonal Parameters

<table>
<thead>
<tr>
<th></th>
<th>E2 pg/ml</th>
<th>FSH mlU/ml</th>
<th>LH mlU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>91.86±4.92A</td>
<td>0.36±0.05A</td>
<td>0.54±0.05A</td>
</tr>
<tr>
<td>T1</td>
<td>66.53±5.12B</td>
<td>0.19±0.02B</td>
<td>0.25±0.05B</td>
</tr>
<tr>
<td>T2</td>
<td>81.09±2.15C</td>
<td>0.29±0.01AC</td>
<td>0.35±0.07C</td>
</tr>
<tr>
<td>T3</td>
<td>97.3±5.6D</td>
<td>0.33±0.09A</td>
<td>0.50±0.06A</td>
</tr>
<tr>
<td>T4</td>
<td>83.3±8.41C</td>
<td>0.26±0.05C</td>
<td>0.42±0.06D</td>
</tr>
<tr>
<td>LSD0.05</td>
<td>6.61</td>
<td>0.065</td>
<td>0.062</td>
</tr>
</tbody>
</table>
The current results recorded a significant decrease in the level of estrogen hormones, FSH and LH in the T1 treatment compared with the negative control.

Perhaps the reason for this is due to the effect of glucocorticoids on the gonads through a direct effect on the ovaries and testes or through the suppression of the hypothalamic-pituitary-gonadal axis, as glucocorticoids act at different levels and suppress both the hypothalamic gonadotropin-releasing hormone hormone (GnRH) and gonadotropin secretion and inhibit the production of testosterone and estrogen by testis and ovary, respectively. On the other hand, glucocorticoids suppress the secretion of adrenocorticotropic hormone (ACTH) in the pituitary gland, which in turn leads to decreased production of androstenedione, which may reduce the secretion of testosterone and estrogen from the adrenal gland [10,11,12].

Glucocorticoids reduce LH secretion and inhibit FSH action in the granulosa cells of the rat ovary through the inhibitory effect of glucocorticoids on gonadotropin, which reduces the production of FSH and LH and reduces the production of gonadotropins [13]. These results are similar to the results of the studies [14,15].

Treatments T2, T3 and T4 of the second experiment witnessed an increase in the level of estrogen hormones, FSH and LH compared to treatment T1. The increase in the level of estrogen hormones may be due to the estrogenic effect of lignan due to its structural similarity with estrogen at the molecular level and due to its low molecular weight and stable structure phytoestrogen as they pass through cell membranes and interact with estrogen receptors conferring the ability to act through intracellular estrogenic pathways [16,17,18].

It was also found that consuming flaxseeds reduced the growth of tumors in mice whose ovaries had been removed, as consuming a diet rich in phytoestrogens for long periods stimulated the growth of estrogens [19].

The increase in the level of FSH and LH hormones after treatment with phytoestrogen may be due to the effects of phytoestrogen on the hypothalamic-pituitary axis, as phytoestrogen contain biologically active
compounds that can affect the hypothalamus to secrete GnRH, which stimulates the pituitary gland to secrete FSH hormones, and LH [20].

The results agreed with [21], as they found an increase in the level of estrogen hormones, FSH, and LH in female rats at the age of menopause (18-20) months after they were fed on a diet containing 15% flaxseed powder for 60 days. The results also agreed with the results of [22] of study, which showed an increase in estrogen hormone in female rats induced osteoporosis with anastrazol after being treated with phytoestrogen (genistein) at a dose of 20 mg/kg of body weight for 60 days.

5. Conclusions

Treatment with Prednisolone caused osteoporosis and negative effects on hormonal parameters, while treatment with phytoestrogen showed improvement in hormonal parameters. Therefore, medicinal plants and their extracts have become one of the best therapeutic methods for many diseases that affect the human race.

6. Acknowledgements

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Reference


