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Effect of Hormone Replacement Therapy on Osteoporosis and Its Complication on Adults Female Albino Rats

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ABSTRACT: This study was conducted to show whether hormonal replacement treatment would offer ameliorating effects against abnormal alterations associated with osteoporosis in female albino rats. Two experiments were carried out, in the first experiment, the rats were randomly divided into two main groups. The first group, were let without surgery and served as control while, the second group were obeyed to bilateral ovariectomy surgery to induce osteoporosis; after 4 weeks induced acute hormonal disturbance indicated by decreased estradiols and progesterone levels. While, the level of follicular stimulating hormone and luteinizing hormone were increase in osteoporosis female rats regarding to levels in normal control female rats. In the second experiment, a remarkable correction was occurred in the levels of follicular stimulating hormone and luteinizing hormone by gradually decreased after 90 days of treatment. While, a considerable amelioration effect was occurred in the estradiols level by gradually increasing of levels with the time and treatment with estrogen replacement. These corrections were dependent on the time of treatment (30, 60 & 90 days). According to the pre-mentioned results, it is importance to concluding that the treatment with estradiol replacement ameliorates chance of recovery from hazardous effects of osteoporoses. The obtained data were discussed according to available obtained researches.

KEYWORDS: Hormonal treatment, Osteoporosis, female albino rats, estradiol, ovariectomy.

1 INTRODUCTION

Osteoporosis is a skeletal disorder characterised by loss of bone mass and deterioration of micro-architecture, leading to bone fragility with a consequent increase in risk of fracture[1],[2]. It is the most common global bone disease, and is a risk factor for fracture of a magnitude similar to the way that hypertension is a risk factor for stroke [1]. It is estimated that approximately 50% of women >50 years' experience an osteoporosis-related facture at some point in their lifetime [1], [3] The most common fractures are those of the vertebrae, proximal femur and distal forearm. Vertebral fractures are experienced by 1 in 8 patients >50 years, of which two thirds are clinically silent [1], [3], [4]. They are associated with a high risk of future fracture, morbidity and mortality. Hip fractures, which affect 1 in 3 women >80 years, are among the most devastating results of osteoporosis [4]. They are associated with a 36% increase in mortality within one year. Complications include emboli, pneumonia and a 2.5 fold risk of future fractures [1], [3]. Up to 20% of patients who experience a hip fracture require long-term nursing care and only 40% of patients fully regain their pre-fracture level of independence. Osteoporosis represents a major public health problem. For example Irish figures indicate that approximately 300,000 people ≥50 years have osteoporosis and that these figures may double in the next 20 years [3]. Osteoporosis-related fractures carry a heavy economic burden; it is estimated that in Ireland, osteoporotic fractures cost up to €551 million to treat in 2010 and will cost more than €1 billion by 2020 [3].

In particular, there are three clinically relevant indications for utilizing bone markers, first, to monitor bone loss in the postmenopausal period, second, to assess overall fracture risk and third, to monitor response to therapy [5]. Menopause,

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whether natural or surgically induced, is associated with elevated levels of circulating total cholesterol and LDL cholesterol, placing postmenopausal women at greater risk for osteoporosis [6]. According to observational studies, up to 50% of osteoporosis in postmenopausal women could be prevented by postmenopausal hormone replacement therapy (HRT). However, a recent randomized secondary prevention study was not able to confirm these results. The hormone is also the principal determinant of the bone remodeling activation threshold, and hence of the amount of remodeling in the total skeleton. estrogen replacement therapy (ERT) remains controversial. On the one hand, hormone replacement seems to play a role in the prevention of osteoporosis and heart disease, and the reversal of some aspects of neurological decline [7], [8], [9].

The Food and Drug Administration (FDA) guideline has appropriately designed the need for rat experimentation in the preclinical evaluation of agents used in the prevention or treatment of postmenopausal osteoporosis. The ovariectomized rat is an excellent preclinical animal model that correctly emulates the important clinical feature of the estrogen depleted human skeleton and the response of therapeutic agents. Its site-specific development of cancellous osteopenia/osteoporosis is one of the most reproducible biologic responses in skeletal research [10].

2 MATERIAL AND METHODS

2.1 MATERIAL

This study was carried out on female albino rats ***Rattus rattus*** as an animal model for induction of osteoporosis. 30 adult female albino rats were employed in the current study. Animals were allowed ten days pre-experiment period to adapt to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in metallic cages at 28±20C and 50% relative humidity and received food and water ad-libitum with fresh supplies presented daily.

2.2 METHODS

The current study was included two experiments. In the first experiment, the rats were randomly divided into two main groups. In the first group, ten rats were let without surgery and served as control while, the second group of twenty rats were obeyed to bilateral ovariectomy surgery to induce osteoporosis as described by [11], [12].

The first experiment was conducted for 4 weeks to facilitate the development of osteoporosis occurrence. At the end of four weeks, a comparison was occurred between five normal control rats and another five ovariectomy rats to evaluate the osteoporosis occurrence due to the disturbance in hormonal pattern.

In the second experiment, osteoporosis rats were further divided into three equal subgroups (five rats for each). They were received subcutaneous injections of 2.0 mg 17-ß-estradiol-3-benzoate (Sigma Chemical, St. Louis, MO, USA)/ 100 g B.wt. in 100 µl sesame oil every 4 days between 10:00 and 11:00 a.m. for three months to simulate estrus cycles. control injections were 100 µl sesame oil. Dosing began at the 5th week after the surgeries. However, the dose of 2.0 mg estradiol in 100 µl reportedly produces plasma estradiol levels similar to peak levels occurring during the ovarian cycle in intact rats [11], [12].

Bilateral ovariectomy surgery was performed in anesthetized with sodium pentobarbital, hair around the skin area where the incision was planned was shaved, and skin was scrubbed with 70% alcohol. Surgery in female rats was making bilateral dorsal abdominal incisions through the skin, such that the ovary and oviduct could be rapidly removed. The success of the bilateral ovariectomy procedure was confirmed at the end of the study by measuring serum estradiol [11], [12].

At the end of each experimental period, rats were overnight fasted, killed by decapitation. Blood samples were collected using polyethylene tubes, blood samples were taken from each rat within each group into clean and dry screw capped centrifuge tubes and left to clot at room temperature, then been centrifuged at 3000 rpm for 15 minutes in order to separate clear serum samples and divided into small aliquots to avoid the effects of repeated thawing and freezing. All sera were stored at - 20°C until used for determination of hormonal profile.

2.3 Biochemical methods

Serum rat follicle stimulating hormone (FSH), luteinizing hormone (LH), and Progesterone were estimated according to [13], [14] & [15] respectively using commercial kits purchased from Isotope Co., Budapest, Hungary. Estrone (E_1) was estimated according to [16] using commercial kits purchased from Fuji Rebio Diagnostics, Inc. U.S.A. Estradiol (E_2) was estimated according to [17] using commercial kits purchased from Diagnostic Products Corporation (DPC), Los Angeles. California. U.S.A. Estriol (E_3) was estimated according to [18] using commercial kits purchased from IBL- Company, U.S.A.

2.4 Statistical analysis

All recorded data were analyzed by applying the following mathematical principles, two-way analysis of variance (F-test) followed by Duncan's multiply range test [19], [20].

3 RESULTS

The current study was carried out to determine the possible therapeutic role of estrogen replacement treatment (ERT) in terms of amelioration of the common side effects of postmenopausal osteoporosis, which induced in female albino rats by removing their ovaries. The study was extended to determine the hormonal profile in normal female rats as well as ovariectomized female rats to find the various, significant changes and relationships between these parameters.

At the first experiment, The levels of estimated hormones were significantly decreased. The mean values recorded were 16.99 ± 0.89, 40.01 ± 1.90, 24.06 ± 1.29 and 7.03 ± 0.49 ng/ml for estradiol's (E1, E2, E3) and progesterone levels in osteoporosis female rats regarding to 30.87 ± 1.69, 113.69 ± 3.71, 43.15 ± 2.41 and 12.19 ± 0.77 ng/ml levels respectively in normal control female rats.

While, the levels of follicular stimulating hormone (FSH) and luteinizing hormone (LH) were increase ($P<0.001$) significantly in osteoporosis female rats. The percentages of these increment were 59.16 and 48.94 for FSH and LH levels respectively as compared to their normal control rats group table(1).

Table 1. The mean values of hormonal profile levels in normal and ovariectomized female rats.

Parameters \ Groups		Control	Osteoporosis
LH uIU/ml	Mean±SD	4.09 ± 0.13	6.13 ± 0.34*
	%		48.94
FSH uIU/ml	Mean±SD	5.73 ± 0.16	9.12 ± 0.61*
	%		59.16
Progesterone ng/ml	Mean±SD	12.19 ± 0.77	7.03 ± 0.49*
	%		- 42.09
E_1 ng/ml	Mean±SD	30.87 ± 1.69	16.99 ± 0.89*
	%		- 43.27
E_2 ng/ml	Mean±SD	113.69 ± 3.71	40.01 ± 1.90*
	%		- 65.48
E_3 ng/ml	Mean±SD	43.15 ± 2.41	24.06 ± 1.29*
	%		-45.16

In the second experiment, the mean value of LH and FSH levels were elevated in ovariectomized rats and recorded 5.41 ± 0.27 and 6.91 ± 0.32 uIU/ml after 30 days regarding to 4.04 ± 0.13 and 5.29 ± 0.16 in normal control rats respectively. Such elevation gradually decreased after 90 days of treatment and reached 4.41 ± 0.31 and 5.66 ± 0.21 uIU/ml respectively at the last interval.

However, two way analysis of variance (F- test) followed by Duncan's multiply range test was revealed a significant interaction between the mean values of the level of LH and FSH at different times in treated groups and those levels alter different times of treatment.

On the other hand, The mean value of E_1, E_2 ,E_3 and Progesterone levels were depleted in ovariectomized rats and recorded 21.43 ± 1.14, 53.19 ± 2.17, 25.71±1.43, and 8.04 ± 0.66 ng/ml after 30 days regarding to 32.04 ± 1.75, 117.75 ± 3.84, 43.93±2.42, and 11.81 ± 0.77 in normal control rats. Such depletion gradually increased with the time and treatment with estrogen replacement and reached to 26.29 ± 1.52, 83.74 ± 2.59, 34.76± 1.78 and 10.13 ± 0.68 ng/ml at the last interval 90 days (table 2 and figure 1).

The data were subjected to statistical evaluation using two way analysis of variance followed by Duncan's multiply range test which revealed a significant interaction ($P< 0.001$) due to the main affects, intervals of estimation, dose of estrogen replacement administration.

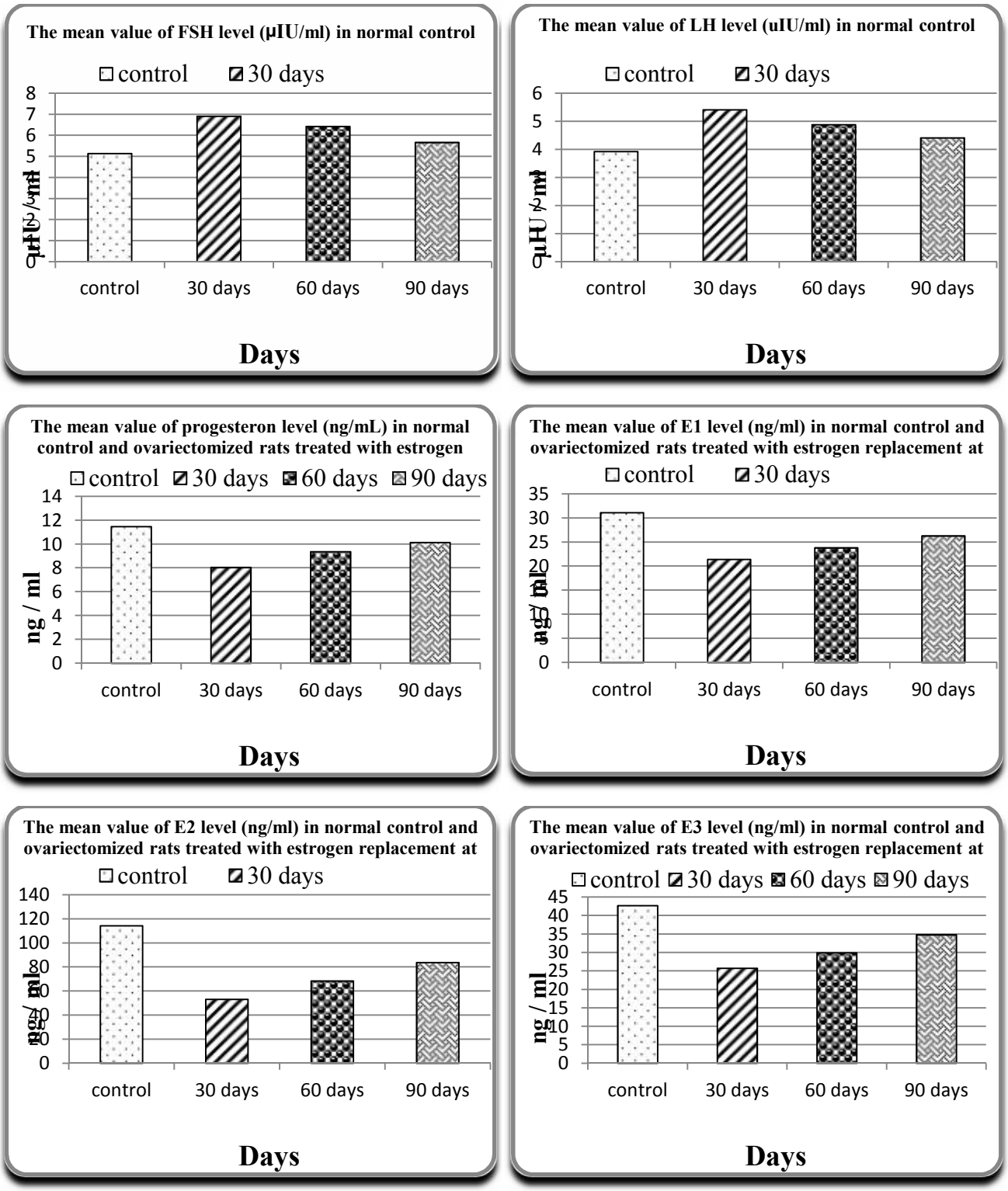

Fig. 1. The mean values of hormonal profile in normal control and ovariectomized rats treated with estrogen replacement at various time intervals

4 DISCUSSION

Osteoporosis is a disease characterized by a decrease in bone mass (osteopenia) and a deterioration in bone micro-architecture which leads to an enhanced fragility of the skeleton, and therefore to a greater risk of fracture. The study group of the World Health Organization has qualified this definition as to state osteoporosis is present when the bone mineral density or bone mineral content is over 2.5 standard deviation below the young adult reference mean. If fractures are present, the condition is known as “severe” osteoporosis [21], [22].

Following ovariectomy, rapid loss of cancellous bone mass and strength occurs, which then proceeds in a less rapid rate in a site-specific fashion to reach steady state phase of bone mass with an increase in rate of bone turnover following oophorectomy or menopause in humans. Not all cancellous bone sites in the rat exhibit such bone loss nor do all cancellous bone sites lose bone at the same rate [23].

These bone loss features mimic the bone changes. The loss of ovarian hormones causes an increase in bone turnover and results in a negative bone balance due to relatively high bone resorption rate compared to bone formation rate, which is also enhanced [24]. Several lines of evidence suggested that a high rate of bone turnover causes great loss of bone [25].

After the mild age or perhaps beginning earlier, bone loss occurs and is accentuated by estrogen deficiency. The protective role of estrogen against bone loss has been proved. FSH and LH may modulate the effect of estrogen in bone, perhaps by activating the estrogen receptor [26].

In the female, the ovaries produce two groups of steroid hormones, estrogens and progesterone. Estrogens, including estradiol, estrone, and estriol, are extremely important in the development of secondary sex characteristics and regulation of the menstrual cycle. Estrogens also influence libido and the metabolism of electrolytes and nitrogen, and help maintain pregnancy and prepare the breasts for lactation. Progesterone, which resembles estrogen chemically, helps regulate changes that occur during menstruation and influences the development of fetal membranes and mammary glands during pregnancy. Three gonadotropic hormones produced by the pituitary gland regulate the secretion of estrogens and progesterone: follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin [27].

In the current investigation, a significant decrease in the levels of estrogens hormones (E_1, E_2 and E_3) and progesterone was occurred in ovariectomy rats. While, a significant elevation in the levels of FSH and LH was occurred in ovariectomy rats. These results may be attributed to the disturbance in the hypothalamus-pituitary-gonadal axis (HPGA) or/ and elevation in the gene expression of insulin growth factor-1 (IGF-1), resisting and neuropeptide hormone (NPY).

This severe deficiency of circulating estradiol in the osteoporotic group may reflect the role of estradiol in regulating bone remodeling. This explanation is depending on several facts, that, estrogen is one of the hormones among several factors regulates the bone remodeling, estrogen deficiency may be an etiological agent for postmenopausal osteoporosis, and can also alter the balance of multiple growth factors and cytokines that regulate bone turnover leading to increase bone resorption than bone formation [28].

Depending on the well-known findings that postmenopausal women with higher estradiol concentrations appear to have greater bone density. Therefore, the dramatic decrease of estrogens hormones (E_1, E_2 and E_3) and progesterone in the ovariectomy rats in the current investigation may increase the risk of osteoporosis.

Hormone therapy (HT) is based on the idea that the treatment may prevent discomfort caused by diminished circulating estrogen and progesterone hormones. It involves the use of one or more of a group of medications designed to artificially boost hormone levels. The main types of hormones involved are estrogens, progesterone or progestin's. It often referred to as "treatment" rather than therapy [29], [30], [31].

The Journal of the American Medical Association and elsewhere based on these findings warn that women with normal rather than surgical menopause should take prescribed HRT treatment at the lowest feasible dose, for the shortest possible time. For health problems associated with menopause such as osteoporosis (a small percentage of postmenopausal women are at risk of severe bone loss), other life-style changes and/or medications are now recommended [32], [33].

HRT may help to prevent or delay the development of many diseases, including the following: osteoporosis, Alzheimer's disease, colon cancer, macular degeneration (the leading cause of visual impairment in persons over age 50), urinary incontinence and skin aging [34], [35].

In the second experiment of this study, the treatment of ovariectomy rats with intrascapular subcutaneous injections of 2.0 mg 17 ß-estradiol-3-benzoate for three months led to a considerable correction in all investigated parameters dependent on the time of administration.

The benefits of hormone replacement therapy include: (1) Controlling menopause symptoms, (2) Preventing heart disease, (3) Preventing osteoporosis, (4) Preventing some hard-to-detect female cancers and (5) Other good reasons. Major studies have reported that women who take estrogen after menopause experience fewer bone fractures than women who do not [34], [35].

5 CONCLUSION

In conclusion, menopause is a normal part of aging. It is not a disease or something that has to be treated. Women may decide to use menopausal hormone therapy because of its benefits, but there are also side effects and risks to consider. Two benefits of menopausal hormone therapy are treating some of the bothersome symptoms of menopause and preventing or treating osteoporosis. Hormone replacement therapy (HRT) can help protect women against osteoporosis. HRT decrease hot flashes, night sweats, decrease the development of hair on the face, a loss of muscle tone in the bladder and urethra, skin changes and improve the mode of sleeping in night.

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