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Evaluation of reproductive hormones and histological of ovary in female rats treatment with dexamethasone

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Abstract

The present study aimed to investigate the effect of dexamethasone Concentration on reproductive hormones and histochange of ovary in female's rats. Twenty four adult female rats (*Rattus norvegicus*) were used. They were divided into four groups, (6) rats for each group. The first group (A) treated with normal saline for three months as a control group, the second group (B) treated with low dose dexamethasone (10 mg/kg/day) for three months, the third group(C) treated with intermediate dose dexamethasone for three months (20 mg/kg/day), and the fourth group (D) treated with high dose dexamethasone (40 mg/kg/day) for three months. The result indicated a significant increase in FSH and LH levels in all treated groups with dexamethasone (B, C and D) groups compared with control group. Also, there was a significant increase in FSH and LH levels in group B compared with C and D groups, with a significant increase in C compare with D (table 1 and 2). The results of histological change in ovary showed effect on all the follicles stimulation in group(B,C) treated with low doses and mid doses compare with control group and hyperplasia of theca and granulosa cells of ovarian treated with high doses groups (D) sections for three month, when compared with control group which show normal histological features . The study concludes that the abuse of dexamethasone caused side effect to woman which used it.

Keywords: Dexamethasone, Rattus norvegicus, FSH hormone, LH hormone, ovary.

تقييم الهرمونات التناسلية والتغيرات النسيجية للمبيض في إناث الجرذان المعالجة بالدكساميثازون

رنا طالب الموسوي

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الخلاصة

تهدف الدراسة الحالية لمعرفة تأثير جرعة الدكساميثازون على الهرمونات الانثوية التكاثرية والتغيرات النسيجية للمبيض في إناث الحيوانات المختبرية. استخدمت في هذه الدراسة 24 جرذ من إناث الحيوانات البالغة وقسمت إلى أربعة مجاميع بواقع ستة جرذ لكل مجموعة ,جرعت المجموع الأولى من المحلول الفسيولوجي وتركت كمجموعة سيطرة.إما المجموعة الثانية فقد عوملت بجرعة قليلة 20ملغ/.كغم/.يوميا) من الدكساميثازون واستمر التجريع لثلاثة اشهر, والمجموعة الثالثة عوملت بجرعة متوسطه 20ملغ/.كغم/.يوميا) من الدكساميثازون لمدة ثلاثة أشهر. إما المجموعة الرابعة فقد عوملت بجرعة عالية الدكساميثازون40.ملغم/.كغم/. يوميا.

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أظهرت النتائج في الدراسة الحالية إن هنالك فرق معنوي $p\leq0.05$ في المجاميع المعاملة بالجرع المختلفة للدكساميثازون مع مجموع الكونترول حيث لوحظ زيادة في مستوى هرمون FSH وLH عن مجموعة السيطرة كما ان هنالك زيادة في مستوى الهرمونات في المجموعة الثانية (B) المعاملة بالجرعة الواطئة (10ملغم/ كغم /يوميا)من الدكساميثازون ولمدة ثلاثة اشهر عند مقارنتها مع بقية المجاميع(C,D) كذلك هنالك زيادة معنوية في مستوى الهرمونات في ألمجموعه (C) عند مقارنتها مع المجموعة (D). إما فيما يخص التغيرات النسيجية ففي الدراسة الحالية وجد هنالك تغيير في جميع المجاميع المعاملة بالدكساميثازون عند مقارنتها في مجموعة السيطرة حيث وجد هنالك زيادة في تضخم الخلايا الحوصلية وحصول نضوج للبيضة وتحول الخلية الثانوية للبويضة الى خلية ناضجة ووضوح الغلالة الحوصلية ووضوح granulosa cells وزيادة نضوج البيوض خاصة في المجموعة الثانية التي عوملت بجرعة منخفضة من الدكساميثازون .من الدراسة الحالية يمكن استخدام الدكساميثازون والاستفادة منه حسب الجرعة المعطاة وعند الإطالة الى فترة طويلة الامد فانه يظهر اثار جانبية لالساءة الاستخدام عند بعض النساء حيث يبدا تغيرات في مبيض الإناث من سمك الحويصلات المبيضية وتحلل البيوض وغيرها كما في المجموعة الرابعة التي استخدمت فيها جرعة عالية من الدكساميثازون ولفترة طويلة.

Introduction

Most of chemical drugs have side effects on various parts of body. It is necessary to identify these effects to better use of drugs. Dexamethasone is one of the most usual drugs in human and animal medication. (Gooyande, 2014).glucocorticoids have been shown to exert direct effects on ovarian steroidogenesis, both *in vivo* and *in vitro* (VanMerris *et al*., 2007). Another mechanism by which the hypothalamic-pituitary-adrenal axis may influence reproductive function is by a direct effect of glucocorticoids on the target tissues of sex steroid production (Hung and Shirley, 2001).

Under the action of the ovarian hormones (estrogen and progesterone) produced by the stimulus of the adenohypophysis, there are clinical structural changes in the endometrial that constitutes the menstrual cycle (Gartner and Hiatt, 1999).

Studies have demonstrated that the glucocorticoids, among which the dexamethasone, inhibit the cellular proliferation and its effects can be observed in the reproductive functions because it blocks several induced responses by the estrogen in the uterus of rats (Szego &Roberts, 1953; Szego & Davis, 1969; Campbell, 1978 and Bigsby and Cunha, 1988). However, Batista *et al.* (2004) reported dexamethasone effect on the level of steroids hormones mainly estrone, estradiol and testosterone and lead to increase their levels in body, which cause the induction of aromatase enzyme (Iida *et al.*, 1991),and also increases the estradiol, testosterone and rostenedione level in both plasma and ovaries cells(Illera *et al*., 2005). However, Batista *et al.* (2004) report the administration of dexamethasone for five consecutive days in induced rats to the endometriosis was not enough to reduce the implant, suggesting that maybe the dosage or the period of the treatment have influenced in the result .Thus the present research aimed to evaluate the effect of dexamethasone in reproductive hormones and histochange of ovary in rats.

Materials and Methods

The study was conducted in Thi Qar University, Collage of science, Department of Biology, 24 female Rat from *Rattus norvegicus* and in weight range of 200±250 g were prepared and kept for two weeks in similar condition with free access to food, water, normal light and appropriate temperature and moisture. These favorite conditions were continued for whole period of study. Rats were divided into four groups with six for each group. The first group (A) was a control group treated orally with normal saline for three months, the second group (B) was treated with low dose (10mg/kg/day) of dexamethasone for three months. The third group (C) treated with intermediate dose (20 mg/kg/day) of dexamethasone for three months. The fourth group (D) treated with high dose (40 mg/kg/day) of dexamethasone for three months.

Specimens Collection

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At the end of experiment the rats were generally anaesthetized by inhalation of chloroform and then sacrificed. Blood samples were collected directly from the heart by the use of disposable syringes of 10 ml capacity, and the blood samples were poured into test tubes free from anticoagulant to isolate blood serum and allowed to clot at room temperature and then centrifuged at velocity (3000 rotation/minute) for (15 minutes) to isolate blood serum and froze at -20 centigrade to estimate measure FSH, and LH hormones. FSH, LH levels were measured using the Micro particle Enzyme Immunoassay method.

Histological methods

The ovarian tissue was fixed in 4% formaldehyde for sample preservative, then the tissue was dehydrated by passing it through increasing concentrations of ethyl alcohol (from 0 to 100%) this step called (processing), after replacement occurs, the alcohol was replaced with xylene, which is miscible with alcohol. This step is called clearing. Then the tissue was embedded in paraffin wax which becomes harden, after which sections of 5 µm diameter were obtained by using rotary microtome. The sections were rehydrated by passing through xylene, and then decreasing strengths of alcohol (100 to 0%) and finally water, and stained with Heamoxlyin and Eosin (H and E) and then dehydrated again using xylene, finally mounted on the microscope slide then cover slip was placed on top to protect the sample and examined under microscope X40(Fischer et al., 2008).

Statistical Analysis

Standard analysis of the data of different studied groups was performed using the computerized statistical program: The SPSS program (Statistical Program for Social Sciences). The results were expressed as mean± standard Error (x± S.E). Analysis of variance (ANOVA) was used to compare the results of different groups.

The differences are considered to be significant at the level 0.05($P \leq 0.05$).

Results

FSH and LH levels

The results indicated as a significant increase in FSH and LH levels in all treated groups with dexamethasone (B, C and D) groups compared with control groups. Also, there was a significant increase in FSH and LH levels in group B compared with C and D groups with a significant increase in C compared with D (table 1 and 2).

(Table 1) Effect of dexamethasone on the FSH level in female rats

Animal groups	FSH level (mLU/dl)
Group A (N.S)	1.42 ± 0.04^{d}
Group B(10mg/kg)	5.77 ± 0.37^{a}
Group C (20mg/kg)	3.91 ± 0.21^{b}
Group D(40mg/kg)	3.50 ± 0.03^{c}
LSD	0.4

Values are means ± S.E. different letters refer to significant differences at ($p\leq 0.05$)
Same letters refer to no significant differences at ($p\leq 0.05$)

(Table 2) Effect of dexamethasone on the LH level in female rats

Animal groups	LH level (mLU/dl)
Group (A)(N.S)	0.13 ± 0.02^{d}
Group B (10mg/kg	4.68 ± 0.95^{a}
Group C (20mg/kg)	3.10 ± 0.04^{b}
Group D (40mg/kg)	2.06 ± 0.01^{c}
LSD	1.03

Values are means ± S.E. different letters refer to significant differences at ($p\leq 0.05$)
Same letters refer to no a significant differences at ($p\leq 0.05$)

Histological results

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Treatment of female rats with low, intermediate doses of dexamethasone for 90 days showed the effect on the follicles stimulation in groups treated (B.C) compared with control group. Also, presence of rats treated with high doses of dexamethasone (group D) compared with control group which showed normal histological features (figure 1).

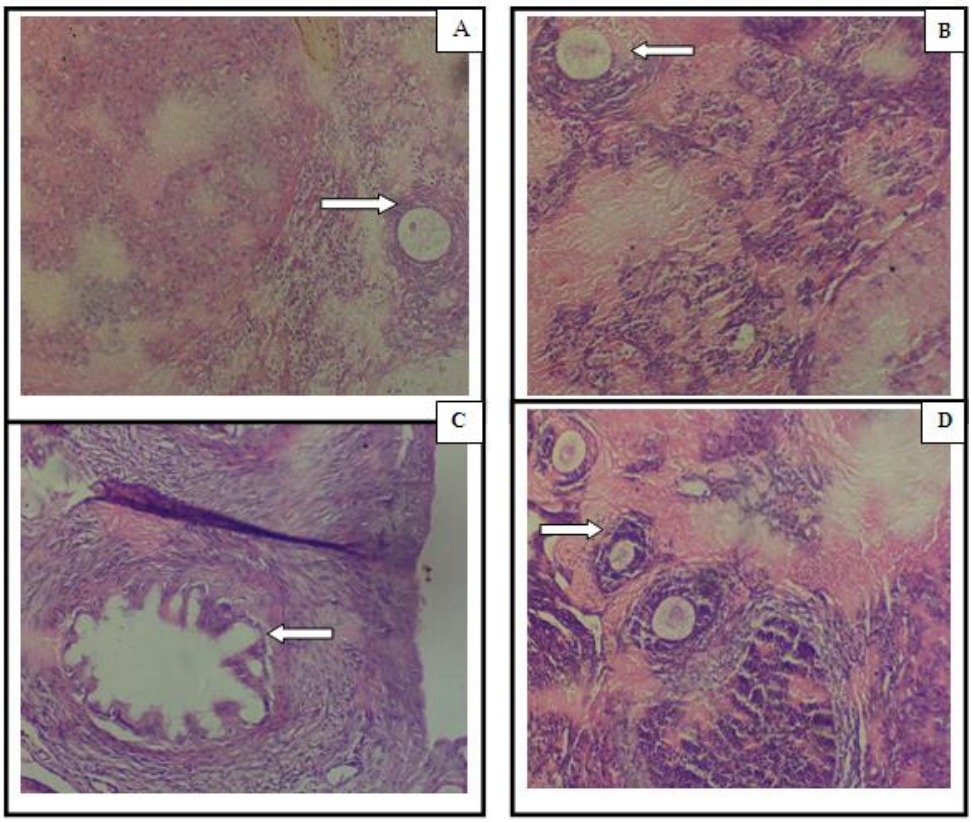

Figure1. Histological sections of ovaries stained with (H&E), examined under microscope (X40). (A)Representative untreated normal section considered as control group compared with section (B, C, D) which treated with doses (10, 20, 40 µg/kg/day). This also explains effect on all the follicles stimulation (B,C) groups and hyperplasia in both granulosa and theca cells and detachment in oocytes in (D)groups .

Discussion

The present study indicated the effect of dexamethasone in femal rats by increasing level of FSH and LH and histochange in ovary these change depended on doses of dexamethasone. Glucocorticoids such as dexamethasone can stimulate FSH synthesis by fatty luminal (steroidprecursors and hormones), and hyperplasia of theca and granulosa cells of ovarian of influencing genes of FSH (Rosen *et al* 1991). Thus, treatment with glucocorticoids increases pituitary FSH mRNA and pituitary content of FSH [McAndrews *et al.*, 1994], suggesting that glucocorticoids affect FSH at the pituitary level directly rather than via the hypothalamus. It is obvious that multiple

dexamethasone treatment changed the dynamics of pituitary gonadotroph populations and caused an increase in volume density and number per unit area of FSH cells. Nolan and, Levy (2001), therefore the increase in FSH cell number could result of differentiation from undifferentiated pituitary cells or stem cells which are the major source of newly formed pituitary cells under various physiological conditions. In an *in vitro* study on female rats treated by 60-600 ng/mL of dexamethasone, it caused synthesis stimulation and secretion of FSH (Suter and Schwartz, 1985). Phillips and Clarke (1990) reported that Dexamethasone in agreement with this study,1 mg/kg of Betamethasone and 0.1 mg/kg Dexamethasone reduced LH concentration and 1 mg/kg of Dexamethasone increased it.

The histological section of ovary reinforced our finding by previous fact that glucocorticoid receptors have been identified in granulosa cells and glucocorticoids have been shown to exert direct effects on ovarian steroidogenesis, both *in vitro* and *in vivo* (Van Merris *e al.,* 2007). Our results showed hyperplasia of both granulose and theca cells with fatty luminal (with hormones and steroids precursors), confirming increase steroidogenesis in both granulose and theca of ovarian cells. Really, the presenting features of women with high glucocorticoids (Cushing's syndrome) is similar to those observed in patients with the polycystic ovarian syndrome. This powered our finding that abuse of dexamethasone lead to infertility in female which administered this drug for long time (Hung and Shirley ., 2001).The present study found the used of dexamethasone in difference doses and in specialized time increase from FSH, and LH levels also stimulate the follicle of ovary but when used prolonged and abused caused side effect this is as result from present study.

Glucocorticoids can indirectly improve the response of poor responders by increasing serum levels of FSH and LH, In the study of Jenkins and colleagues, following pituitary suppression in IVF cycles, co-treatment with dexamethasone resulted in an increase in serum IGF-1 levels growing follicle (Jenkins *et al* .,1994). On the other hand, dexamethasone improved ovarian responsiveness by reduced effect of adrenal androgens on follicular growth (Isaacs, 1997). In another study by Kemeter *et al* (1986) found higher pregnancy rates in dexamethasone group compared to control. Also, in a study by Keay *et al* (2002), showed that dexamethasone reduced the incidence of poor ovarian response .

In conclusion, this study has shown that use of the dexamethasone with hormone-free interval suppressed pituitary gonadotropins and increased volume and blood flow within the ovary and it also induced from FSH and LH hormone level and we found stimulation of follicle ovum in ovary but did not prolonged in used of dexamethasone because ,it will cause side effect for woman have been used.

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