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Some physiological and histological effects of genistein in female albino rat

بعض التأثيرات الفسلجية والنسجية للجنستين على اناث الجرذان

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Abstract:

The present study was conducted to determine the effects of oral administration of genistein on hematological (total count of red blood cells "RBCs", white blood Cells "WBCs", and packed cell volume "PCV") , hormonal (follicle stimulating hormone "FSH", luteinizing hormone "LH" and estrogen "E") and histological changes in liver and kidney of female rat. Twelve mature female albino rat 10-12 weeks in age, weighting 200-250 g were divided randomly to two groups (6 animals /group). The group was control treated with 1:4 DMSO only and second as the experimental group and received 100 mg genistein/1kg body weight/day dissolved in 1:4 dimethysulfoxid DMSO for 16 days. At the end of experiment, blood samples were collected by heart puncture, then animals were sacrificed and the liver with kidneys was harvested for light microscopy study. The results showed decreased $p \leq 0.05$ RBC count and PCV, whereas WBC count was increased $p \leq 0.05$ in genistein treated group in compared with control grouped. Estradiol level was significantly increased $p \leq 0.05$ while LH showed significant decrease $p \leq 0.05$ in serum of females due to genistein administration. Female rats exposed to high dose of genistein have no obvious histological changes in liver and kidneys. In conclusion, the daily genistein administration caused severe untoward effects on blood and reproductive hormones like other exogenous estrogen analogues.

الخلاصة

اجريت الدراسة الحالية لتعيين تاثيرات الاعطاء الفموي للجنستين، الصورة الدموية (العدد الكلي لخلايا الدم الحمراء "RBCs" خلايا الدم الحمراء "WBCs" وحجم الخلايا المضغوطة "PCV") والهرمونية (الهرمون المحفز للجريبة "FSH" وهرمون اللوتيني "LH" وهرمون الاستروجين "E") والتغيرات النسجية في الكبد والكلى لأناث الجرذان. أثنى عشر انثى جرذان الامهق بعمر 10-12 أسبوع وبوزن 200-250 غرام، قسمت عشوائيا الى مجموعتين (6حيوان/مجموعة). الاولى اعتبرت كمجموعة سيطرة واعطيت DMSO 4:1 فقط اما المجموعة الثانية فانها مجموعة تجربة وجرعت 100 ملغرام جنستين/ 1 كيلوغرام /لليوم اذيبت في 4:1 دايمثيل سلفوكساید DMSO لمدة 16 يوم.

في نهاية التجربة جمعت عينات الدم من القلب مباشرة ثم قتل الحيوان واخذ الكبد والكلى لدراسة المجهر الضوئي.

أظهرت النتائج قلة بخلايا الدم الحمراء وحجم الخلايا المضغوطة، بينما خلايا الدم البيضاء ارتفعت معنويا ($p \leq 0.05$) في المجموعة المعاملة بالجنستين بالمقارنة مع مجموعة السيطرة. هنالك زيادة معنوية ($p \leq 0.05$) لهرمون الاستروجين بينما اظهرت انخفاض معنوي بهرمون LH في مصل اناث الجرذان نتيجة لاعطاء الجنستين. اناث الجرذان المعرضة لجرع عالية من الجنستين لم يشاهد عليها اي تغيرات ملموسة وواضحة في الكبد والكلى. نستنتج الاعطاء اليومي للجنستين يظهر تأثيرات شديدة على الدم والهرمونات التكاثرية كما في نظائر هرمون الاستروجين الخارجية الاخرى.

Introduction

Over the last decades chemicals recorded in the different types of plants that have affinity to the estrogen receptor (ER) and may lead to initiate mechanisms of estrogen action (1). Phytoestrogens are isoflavonoids, diphenolic chemicals detected in the bean subfamily of Leguminosidae (2). Genistein is a soy phytoestrogen and it is isoflavone in nature. Isoflavones are polyphenolic compounds found in variety plant families, It is reported in several legumes such as soy, peanut, green peas, chick peas and alfalfa (3) and (4). Isoflavones concentration in Soy beans are averaged 1-2 mg/gram (5) and represent the important source of dietary isoflavones. Genistein also called

phytoestrogen because of its ability to shows estrogenic activities in vivo, mimic estrogen functions by interacting with estrogen receptor-β (6). Haematological study both in humans and in animal sciences is considered to be an important index of the physiological state of the individual. It has already been proved that the blood picture may undergo huge changes during the life time (7). The blood profile can undergo drastic changes with certain conditions such as, stress, infections and intoxications. Knowledge on the estrogenic and /or anti-estrogenic properties of soy isoflavone genistein, is steadily increasing, however, still far not sufficient and direct action on the blood profile have less reported till date(8). Genistein being a phytoestrogen, and might bind to the estrogen receptors and in turn may affect the haematological and hormonal parameters. Therefore, the main aim of the study was to investigate the effect of genistein on the haematological and hormonal parameters in addition to histological changes in liver and kidney.

Materials and methods

Twelve adult female albino rat were used in this study. They were obtained from the Iraqi national center for drug safety and evaluation .animals aged between 10-12 weeks and the average weight ranged between 200 – 250g they were kept under suitable environment conditions of 20-25c.feed and water were offered daily. The animals were kept for at two weeks for adaptation before experiment start. Adult female rats were used in the present study divided into two groups as following: control group that consist of sex female rat were received DMSO (0.5ml/kg BW) for 16 days and treatment group that consist of sex female rat treated with 100mg/kg BW of genistein dissolved with DMSO orally for 16 days.

At the end of experiment the animal were anesthetized with (ketamine 100mg/ 1kg BW) and (xylazine 100mg/ 1kg BW) and the blood was collected by heart puncture with using 5 ml disposable syringe1ml of blood collected in heparinized tube for hematological tests that were done by using Veterinary Auto-hematology analyzer (Genex Inc., Florida USA).

The rest of the blood put in plane tube to be centrifuged (3000 rpm for 15 minutes) to obtain the serum, which is then transferred to epndrofe tubes, for hormones measurement. Estimation of serum luteinizing hormone (LH) and Follicles-stimulating hormone (FSH) level according to (**10**). Then after, these rats were sacrificed and liver and kidney trimmed out to use for histological examination according to Mescher method (11).

The results were represented as mean± standard error. The data were analyzed by t test and a probability ($p \leq 0.05$) was accepted as significant SPSS 2014

Results and Discussion

The result of the present study revealed significant increase in total leukocyte number in animal treated with genistein in compare with animal of control group (table 1) .

According to Ugochukwu and colleagues (12), estrogen may down regulate the expression of adhesion and chemokine molecules in response to inflammation in many animals. Therefore, it may be one cause behind the increase in the number of WBC after genistein (a phytoestrogen) administration. Researchers have also reported that estrogen treatment alters the recruitment and adhesion of leukocytes to the endothelium, which was induced by inflammation promoters that offer a possible mechanism by which estrogen exert an anti-inflammatory effect (8). These effects of estrogens were due to aiming at the interaction of monocytes with the vascular endothelium (13). The increase in WBC count after exposure to genistein (100 mg/kg body weight) may be one of the basic defense mechanisms of body raised against any exogenous material.

The result also showed significant decrease in RBCs count and PCV in treated group compared with control group (Table 1). The administration of estrogens or diethylstilbestrol has been known to reduce erythropoietin production in rats (14).

Genistein is estrogen-like chemical with possible similar effects to diethylstilbestrol therefore, in the present study the decrease of RBC count, which in turn, resulted in decreasing PCV. May, caused by decrease erythropoietin production due to estrogenic activity of genistein or may be resulted from an increase in destruction of red blood cells.

However, endogenous estrogen has ability to increase metabolism of lipid, erythrocyte sedimentation rate and decrease count of erythrocyte (8). Genistein has known to its estrogenic effects and therefore the decrease in erythrocyte count recorded in this study may resulted from the hyperlipaemia due to genistein administration, similar to that caused by endogenous estrogen (15). Estrogen thus may cause haemodilution by increasing the plasma volume, which may be one of the causes behind the fall in RBC count and PCV (16).

Table (1) shows effect of Genistein on WBCs, RBCs and PCV in female rats (Mean± SE)

Groups / parameters	Total WBCs Count Cell $\times 10^9$/L	Total RBCs Count Cell $\times 10^{12}$/L	PCV %
Control	3.30 ± 0.18	7.58 ± 0.19	41.12 ± 1.04
Genistein	3.95 ± 0.37*	4.69 ± 1.22*	23.37 ± 6.63*

N=6

* represent a significant difference at ($p \leq 0.05$).

Administration of genistein in the present study lead to significant increase in serum estrogen level and decrease in LH level compared with control group a (table 2). The elevation in serum estrogen level may be due to the additional estrogen potency of genistein relative to the existing endogenous estrogen equivalents.

Moreover, bisphenol A (has estrogenic effects resemble that of genistein) interfere with estrogen synthesis pathways via increasing expression level of Cytochrome P450 19a (CYP19a) as recorded by (17). CYP19a is known steroidogenic enzyme act to catalyze conversion testosterone into estradiol and androstenedione to estron in the granulosa cells(18) leading to release of the more endogenous estradiol hormones into circulations.

Decrease of serum LH in the present study could be resulted of estrogenic properties of genistein at the level of the hypothalamic-pituitary-gonad axis. Genistein resemble the estrogenic inhibition of LH secretion but without affect secretion of FSH, (FSH level was unchanged in the present study), suggesting that the effect of genistein on pituitary is specific to the mechanism that control of LH secretion. The reason why genistein has no effect on the FSH secretion, because the control of FSH secretion differs from that of LH secretion (19).

Table (2) shows effect of Genistein on some estrogen, LH and FSH in female rats (Mean± SE)

Groups / parameters	E pg/ml	LH µIU/ml	FSH µIU/ml
Control	51.27±0.42	3.42± 0.36	4.84± 0.28
Genistein	55.37±0.49*	1.56 ±0.64*	4.15 ± 0.13

N=6

* represent a significant difference at ($p \leq 0.05$).

The liver tissue examination of the control group has shown normal histological structure of the liver, the hepatic lobules that consisted of hepatocytes arranged in hepatic cords radiating from the central vein to the periphery of the lobule. The cellular cords separated by sinusoids (Fig 1). The genistein (100mg/kg BW/day) didn't cause obvious changes in liver histology, these results were matched with results obtained by (20) who reported that unaffected liver histoarchitecture in mice when administered 125 and 250 mg/kg/BW

Estrogenic compound have reported to be beneficial to hepatic health and genistein reduce hepatic lipogenesis and inflammation (8) and (9).

Kidney histological structure also appear intact after genistein treatment when compare with control group (fig. 3 and 4), these result may be due to short period of treatment (acute) with genistein.

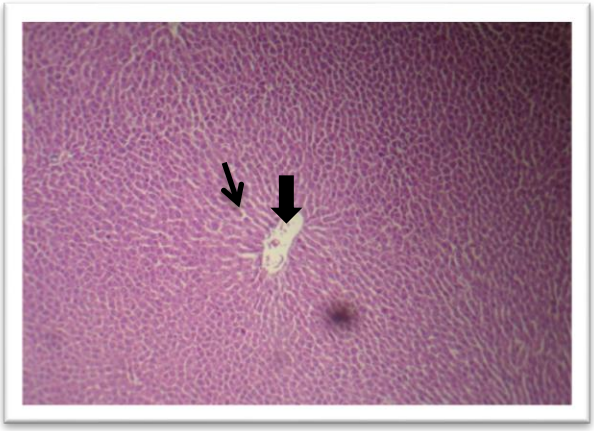

Figure 1: Photomicrograph of a liver section of control rat showing normal histological structure of central vein (thick arrow) and surrounding hepatocytes **(**thin arrow**).** H & E. 10x

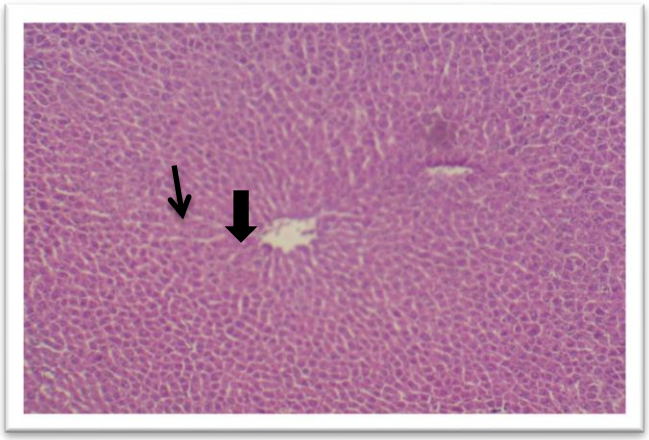

Figure 2: Light micrograph shows the histological structure of liver genistein administered rat in the experiment one shows normal central vein (thick arrow) and hepatocytes (thin arrow) arranged in irradiation manner. H&E, 10x.

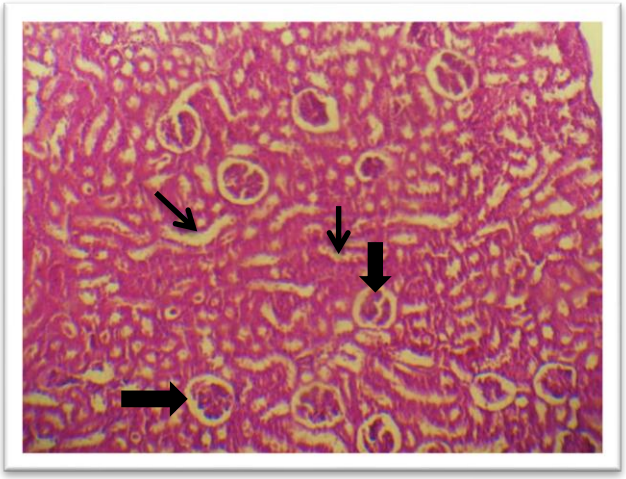

Figure 3: Photomicrograph of kidney tissue of control rat showing normal glomerulus (thick arrows) and tubules (thin arrows).H & E. 10x

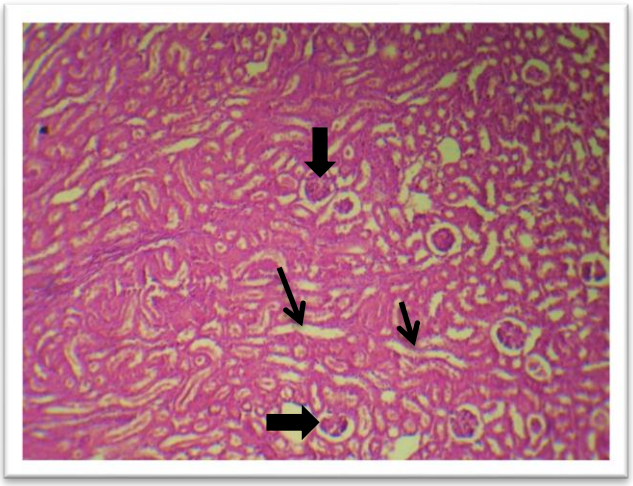

Figure 4: Photomicrograph of a kidney section of rat treated with 100mg/kg/ BW of genistein showing intact renal tubules (thin arrow).and glomeruli (thick arrow) H & E. 10x

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