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Influences of Insulin, n-Acetyl Cysteine, and Folic Acid on the Level of Sex Hormones in the Diabetic Pregnant Rats

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ABSTRACT

The preventive influences of folic acid, N-acetyl cysteine (NAC), and insulin were examined on the alterations of reproductive system related hormones in alloxan-induced diabetic pregnant rats. Healthy pregnant rats (192) were treated with single subcutaneous injection 100 mg/kg body weight alloxan.

The experimental rats were divided into five groups: group one represented negative control, group two was positive control. Group three was treated with folic acid 0.25mg/kg; group four, daily injected with insulin; and last group was treated with NAC 1%. Each of 17- β -Estradiol; progesterone; follicle stimulating hormone (FSH) and luteinizing hormone (LH) were assayed by ELISA.

Alloxan induced diabetic pregnant rats showed significant decrease in all sexual hormone parameters and in all three weeks of gestation. The hormone β-Estradiol none significantly increased in all treatment groups, while significant increasing in progesterone value was observed in all treated groups. Insulin, restore the elevation of FSH and LH hormones except for LH in second week. Treatment with folic acid 0.25 mg/kg was showed significant increase FSH and LH except FSH in third week and LH in second week. While NAC showed less protective effects through decreasing of only progesterone and estrogen levels.

Key word: diabetes, pregnancy, sex hormone

دراسة تأثيرات كل من الانسولين و حامض الفوليك و الاستايل سستين على مستوى الهرمونات الجنسية في الجرذان الحبلى والمحدث فيها داء السكري بالالوكسان

الملخص

تم دراسة التأثير المانع لكل من حامض الفوليك, أستايل سستين و الانسولين على مستوى الهرمونـات الجنسية للجرذان الحوامل المصابة بداء السكري. أشتملت الدراسة 192 جرذا حـاملا خـضعت للظـروف القياسية (12:12ساعة أضاءة و ظلام) وتم استحداث داء السكري بحقن الاناث الحوامل تحت الجلد بجرعـة واحدة من مادة الالوكسان بتركيز 100 ملغم/ كغم من وزن الجسم .

قسمت الاناث الحوامل الى خمسة مجاميع:المجموعة الاولى تمثل السيطرة السالبة, المجموعة الثانيـة هي السيطرة الموجبة, المجموعة الثالثة اضيف 0.25 مايكروغرام/ كغم حـامض الفوليـك الـى غـذائها, المجموعة الرابعة حقنت يوميا باربع وحدات دولية من الانسولين و المجموعة الاخيرة 1%اسـتايل سـستين اضيفت الى غذائها. وتم قياس مستوى كل من هرمون بيتا-اسـترادیول, بروجـسترون, هرمـون المحفـز للجريبات FSH, هرمون الابايضةLH باستخدام عبوة اليزا الجاهزة الخاصه بكل هرمون.

اظهرت مجموعة السيطرة الموجبة انخفاضا معنويا من تركيز جميع الهرمونات مقارنتة مع السيطرة السالبة في الاسابيع الحملية الثلاثة. الانسولين كان له التاثير الفعال على رفع مستوى جميـع الهرمونـات ماعـدا هرمون LH في الاسبوع الثاني للحمل. بينما استخدام حامض الفوليك ادى الى رفع معنوي في تركيز جميع الهرمونات اثناء الحمل ماعدا هرمون LH في الاسبوع الثاني للحمل و FSH في الاسبوع الثالث. بشكل عام لم يظهر الاستايل سستين تاثيرا وقائيا مذكورا على مستوى الهرمونات الاربعة.

INTRODUCTION

Insulin-dependent type (I) diabetes mellitus is characterized by chronically elevated blood glucose levels brought about by a deficiency in insulin production. This elevation of glucose results in serious physiological and pathological complications; in addition, women with poorly controlled diabetes often suffer from reproductive problems, such as spontaneous abortions, neonatal morbidity and mortality, and congenital malformations (Jovanovic *et al*., 1981; Becerra *et al*., 1990; Kelle and Moley, 2005). Work with diabetic animal models were demonstrated uterineatrophy (Hassan *et al*., 1993), reduced mating ability (Farrell *et al*., 2002), and alterations of the hypothalamic-hypophysialovarian axis (Tesone *et al*., 1986). Type I diabetes also leads to lesions in ovarian function (Babichev *et al*., 1994a). Diabetic animals ovulate at a lower rate than animals with normal glucose levels (Powers *et al*., 1996) and also exhibit altered ovarian steroidogenesis (Angell *et al*., 1996), decreased hormone-binding responsiveness (Greene, 1999), and an increased incidence of atresia (Foreman *et al*., 1993).

Estrogen and progesterone might be important in the process of implantation during pregnancy, estrogens increased the blood flow through the uterus; they caused hypertrophy of the uterine myometrium and stimulated breast ductal proliferation (Greenstein, 2001).

Pregnancy is associated with a depression of the immune inflammatory system, and with increased growth and function of the pancreatic islets of Langerhans. The estimation of serum progesterone, oestrogen, human chorionic gonadotropin, and placental lactogen in type 1 diabetic pregnant caused a slight increase in each of serum progesterone, oestrogen and human chorionicgonado tropin (HCG) concentration in diabetic women during the third trimester (Stewart *et al*., 1989). Physiological endocrine changes during normal pregnancy were relatively undisturbed by insulin-dependent diabetes or the degree of diabetes control achieved (Stewart *et al*., 1989). While other investigetors (Reis *et al*., 2002) showed that intrauterine tissues placenta, amnion, chorion, decidua express hormones and cytokines were play a decisive role in maternal-fetal physiological interactions, and they also presented that the excessive or deficient release of some placental hormones in association

with gestational diseases might reflect an abnormal differentiation of the placenta, an impaired fetal metabolism, or an adaptive response of the feto-placental unit to adverse conditions. Since glucose might play different roles in the implantation, it was important to assess how a diabetic environment would influence sex hormone fluctuations during gestation.

The teratological processes in diabetic pregnancy are not completely understood. In recent years, however, a putative excess of reactive oxygen species (ROS) has been observed in studies during which diabetes-induced embryopathy was blocked by antioxidants *in vitro* and *in vivo* (Kinalski *et al*., 2001).

Since oxidative stress is an important pathway for fetal injury diabetic mother administration of folic acid can diminish diabetes-induced maldevelopment. N-acetyl cysteine (NAC) is a thiol containing antioxidant that either increases intracellular glutathione concentrations (an endogenous reducing agent) and/or acts directly as a free radical scavenger (Lappas *et al*., 2003; Wentzel *et al*., 2005)**.**

MATERAILS AND METHODS

Adult female albino rats *Rattus norvegicus* bred in the animal house of Biology Dept. /College of Science/University of Salahaddin. In the present study 192 healthy pregnant rats weight about (200-250) gram were used. The animals were housed in plastic cages bedded with wooden chips. The animals were housed under standard laboratory conditions 12 h light: 12 h dark photoperiod, 22±2 C^{o} (Coskun *et al*., 2004). The animals were given standard rat pellets and tap water and libitum.

Diabetes was induced by a single subcutaneous injection of 100 mg /kg body weight of alloxan monohydrate (BDH Chemical Ltd. England) dissolved in citrate buffer (pH = 4.5) immediately before injection. The control animals received citrate buffer only (Nimenibo-Vadia, 2003). Diabetes mellitus was confirmed in induced rats by testing blood glucose using indicator sticks (Accu-check Roche Diagnostics GmbH, Mannheim, Germany) were considered as alloxan induced diabetes rats (Gidado *et al*., 2005). Symptom of diabetes was observed within three days of alloxan injection.

Normal and diabetic pregnant rats were distributed into three periods of gestation 1-7, 8-14 and 15-22 days.

In all groups, normal female rats about 200-250 gm body weight mated with normal male. Then first day of gestation was detected by vaginal smear for sperm or indication of pregnancy (vaginal plug detection), and then divided to five groups in each week. Group one represented negative control normal pregnant rats, group two is positive control treated with single injection of 100mg/kg alloxan intrapretoneously. Group three, pregnant rats treated with 100mg/kg alloxan intrapretoneously was supplemented by250 mg/kg folic acid in diet. Group four; pregnant rats treated with 100mg/kg alloxan intrapretoneously were injected with 4 IU/Kg insulin subcutaneously daily. Group five; pregnant rats were treated with 100 mg/kg alloxan intrapretoneously only 0.1 % NAC supplemented in their diet daily. Each hormone was assayed with ELISA kit: 17-β-estradiol kit's catalog number: BC-1111; progesterone catalog number: BC-1113; follicle stimulated hormone (FSH) and luteinizing hormone (LH) kit's code: 625-300.

At the end of each experiment, the rats were anesthetized with ketamine hydrochloride 100mg/kg. Blood samples were taken by cardiac puncture; then serum was stored at -80C^0. (Sony, Ultra low, Japan).

Statistical analysis was carried out using statistically available software Microsoft Excel). Comparisons between groups were made using one-way analysis of variance (ANOVA) with Least significant difference as multiple comparison tests. P values < 0.05 were considered significant (Kirkwood, 1988).

RESULTS AND DISCCUSION

The hormonal parameters in alloxan treated pregnant rats during three weeks of gestations were shown in figures (1, 2, 3, and 4). β -Estradiol values were significantly decreased in alloxan treated pregnant rats during three weeks of gestation with mean value of 7.8±1.009, 12.3±1.133, and 12.3±1.133 pg/ml respectively in comparison with negative control with mean values of 70.4±5.116, 75.75±4.958, and 82.75±1.363 pg/ml respectively. The decrease in β-Estradiol insulin depended diabetes during pregnancy agree with literature (Savchenko *et al*., 1991). Diabetes mellitus alteration hypophysary/ gonadal hormonal axis in which is reflected by changes in all these ovary functions, in addition to the pathological changes caused by the metabolic disturbance induced by diabetes itself (Ballester *et al*., 2007). β -Estradiol was none significantly increased in each of treated groups with folic acid 0.25 mg/kg, insulin, and NAC 1%.

Figure (2) showed significant decrease in progesterone value detected in diabetic pregnant rats with mean values of 14.55±1.022, 14.625±0.986, and 15.45±1.704 ng/ml in comparison with negative control with mean values of 43.475±2.179, 49.5±2.072, and 49.5±2.102 ng/ml. Type I diabetes caused alterations of the hypothalamic-hypophysialovarian axis (Tesone *et al*., 1986; Babichev *et al*., 1994 a, and Babichev *et al.* 1994b) and also leads to disturbance in ovarian function (Garris, 1985 and Fernando *et al*. 2002). Significant increase in progesterone value was observed in all treated groups.

The hormone follicle stimulating hormone (FSH) was significantly decreased in diabetes pregnant rats with mean values of 0.573±0.143, 1.228±0.422, and 0.338±0.063 ng/ml in comparison with negative control with mean values of 10.973±0.449, 22.473±0.896, and 5.24±0.496 ng/ml. FSH value significantly increased in insulin treated group during three weeks of gestation. The insulin recovery occured through the normalization of glycemia, also probably via a direct action of insulin on the female reproductive system (Ballester *et al*., 2007). Insulin stimulates the synthesis of FSH in cultured cells, which concomitantly stimulated the in vitro synthesis of estrogens and progesterone (Davoren and Hsueh, 1984). Folic acid 0.25 mg/kg treated group significantly increased FSH value during first and second week of gestation. Folic acid reduced the oxidative stress produced by alloxan, as a result reduced sexual dysfunction and hormone balance created by alloxan (Colton *et al.,* 2002). While in NAC 1%) treated group significant increase in FSH was observed only in first week of gestation, as shown in Figure (3).

Significant decrease in LH value detected in alloxan induced diabetic pregnant rats with mean values of 0.052±0.019, 0.177±0.011, and 0.029±0.001 ng/ml in comparison with normal pregnant rats with mean values of 1.45±0.065, 2.044±0.131, and 0.324±0.014 ng/ml. This result is in agreement with (Bestetti *et al*., 1985 and Ballester *et al*., 2004). They

showed that insulin dependent diabetes caused a decrease in serum levels of FSH and LH in different animal models, which was accompanied by a loss of sensitivity of ovary cells to these two hormones (Katayama *et al*., 1984). During third week of gestation, LH value significantly increased in all treated groups as compared with positive control, as shown in figure (4). Both of folic acid and insulin treated group's significantly increased LH value during first week as compared with positive control of gestation. Potential mechanisms underlying the gonadotropic activity of insulin included direct effects on steroidogenic enzymes, modulation of FSH or LH receptor number (Poretsky and Kalin, 1987).

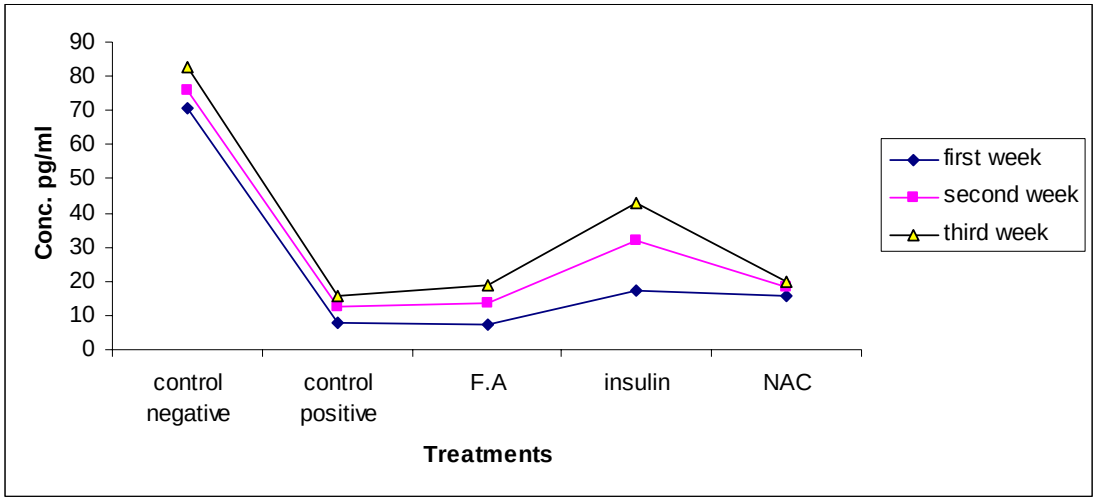

Fig. 1: The effect of folic acid, N-acetyl cysteine, insulin on serum β-Estradiol concentration in alloxan induced diabetes in rats during gestation.

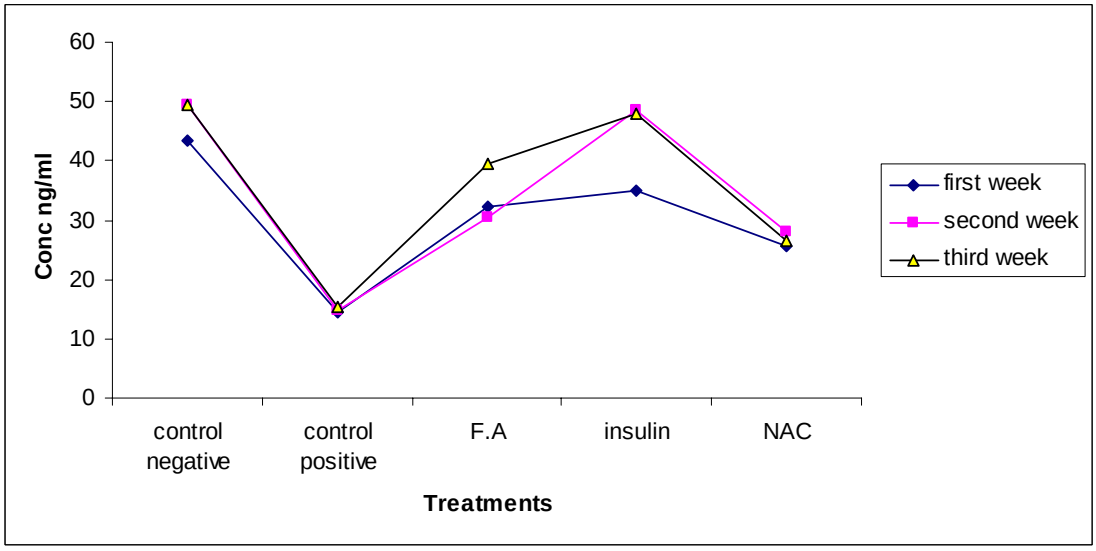

Fig. 2: The effect of folic acid, N-acetyl cysteine, insulin on serum progesterone concentration in alloxan induced diabetes in rats during gestation.

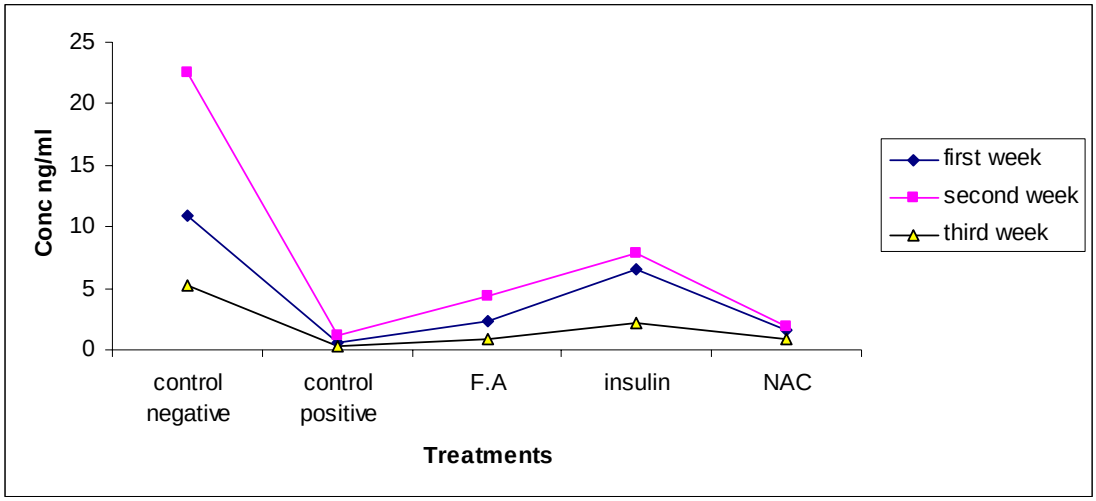

Fig. 3: The effect of folic acid, N-acetyl cysteine, insulin on serum FSH concentration in alloxan induced diabetes in rats during gestation.

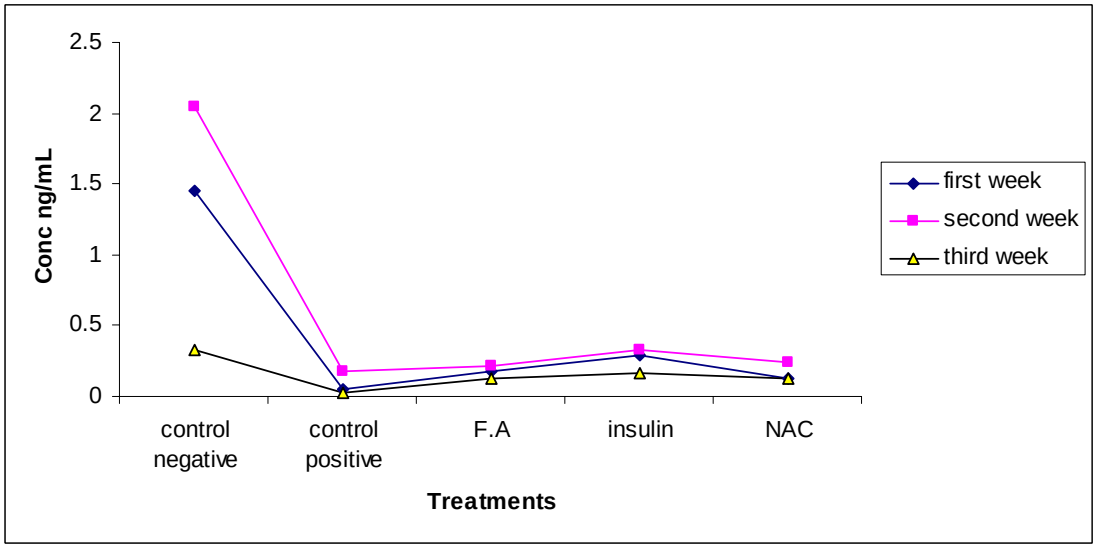

Fig. 4: The effect of folic acid, N-acetyl cysteine, insulin on serum LH concentration in alloxan induced diabetes in rats during gestation.

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