

The protective role of clomiphene citrate against the hormonal disturbances caused by atrazine exposure in adult male rats

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الدور الوقائي لسترات الكلومفين ضد الاضطرابات الهرمونية الناتجة من التعرض للاترازين في ذكور الجرذان البالغة

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المستخلص

هدفت الدراسة الحالية الى اختبار الدور الوقائي لسترات الكلومفين ضد التأثيرات المسببة لاضطراب الهرمونات الناتجة من التعرض للاترازين. استخدم ثلاثين من ذكور الجرذان البالغة قسمت عشوائيا وبصوره متساوية إلى خمسة مجاميع . اعتبرت المجموعة الأولى كمجموعة سيطرة جرعت فمويا الماء المقطر , أما المجاميع الأربعة الباقية فقد جرعت فمويا :- اترازين 50 ملغم / كغم , اترازين 50 ملغم / كغم مع سترات الكلومفين 0.5 ملغم / كغم , اترازين 50 ملغم / كغم مع سترات الكلومفين 0.6 ملغم / كغم , اترازين 50 ملغم / كغم مع سترات الكلومفين 0.7 ملغم / كغم يوميا ولمدة 30 يوم. أظهرت النتائج بأن التعرض للاترازين نتج عنه انخفاض معنوي في مستويات هرمون التستوستيرون , FSH , LH , و هرمون الادبونكتين في المجموعة المعرضة للاترازين مقارنة مع مجموعة السيطرة . نتج عن العلاج الوقائي بسترات الكلومفين (0.6 , 0.7 ملغم / كغم) زيادة معنوية في مستويات هرمون التستوستيرون , FSH , LH , و هرمون الادبونكتين مقارنة مع المجموعة المعرضة للاترازين . أما الاستجابة للجرعة (0.5 ملغم / كغم) من سترات الكلومفين فقد أدت إلى زيادة معنوية في مستويات هرمون LH , و الادبونكتين في حين لم تختلف معنويا عن المجموعة المعرضة للاترازين في مستوى هرمون التستوستيرون و FSH. نستنتج من الدراسة الحالية أن لسترات الكلومفين اثر ايجابيا على مستويات هرمونات التستوستيرون و LH و الادبونكتين .

Abstract

The present study aimed to investigate the protective role of clomiphene citrate against the hormonal disturbances caused by atrazine exposure. Thirty adult male rats were used, they divided randomly and equally into five groups. The first group serve as a control group orally dosed with distilled water , the rest four groups orally dosed with :- atrazine (ATZ) 50 mg /kg b.w. , ATZ 50 mg /kg b.w, and clomiphene citrate (CC) 0.5 mg / kg

b.w. , ATZ 50 mg /kg b.w. and CC 0.6 mg / kg b.w. , ATZ 50 mg /kg and CC 0.7 mg / kg b.w. daily for 30 days. The results showed that atrazine exposure result in significant decrease ($p \leq 0.05$) in serum testosterone, FSH, LH, and adiponectin hormones in atrazine exposed group compared with control group . The protective treatment with 0.6 and 0.7 mg / kg b.w of CC result in significant increase ($p \leq 0.05$) in serum testosterone, FSH , LH , and adiponectin hormones compared with atrazine exposed group . The response to the dose of 0.5 mg / kg b.w. of CC result in significant increase in serum levels of LH , and adiponectin hormones and non-significantly different from atrazine exposed group in FSH and testosterone serum level . It has been concluded from the present study that clomiphene citrate have a positive effects on serum level of testosterone, FSH , LH , and adiponectin hormones.

Introduction

A number of chemicals in the environment have the potential to disturb the endocrine system. These are classified as endocrine disruptors, and many of them are known to have an influence on reproductive potential (1;2) . Atrazine (ATZ) is one of the most widely used agricultural pesticides all over the world, is now recognized to have disrupting effects on the reproductive systems of mammals (3;4). Long-term use of such chemicals give rise to a wide range of reproductive abnormalities including undescended testis, developmental testicular changes and apoptosis of cells in testis (5). Several studies have shown that ATZ produced toxic effects primarily on hypothalamic control of pituitary-ovarian and endocrine function (6). Clomiphene citrate is a well-known selective estrogen receptor modulator that increases gonadotropins secretion via hypothalamic-pituitary action. Clomiphene has already been extensively used in the evaluation of the gonadotropic axis and in the induction of ovulation and it having influences on fertility, In the females (7) and males (8).

Adiponectin , is a protein hormone, secreted from adipose tissue and it is present in the blood in high concentration that accounts for up to 0.01-0.05% of total plasma protein.(9; 10). Decreased circulating adiponectin concentration has been associated with several disorders including: obesity, essential hypertension, insulin resistance, type 2 diabetes, dyslipidemia, cardiovascular diseases and some types of cancer (11). Some studies indicate that adiponectin plays an important role in the inhibition of the inflammatory response and displays anti-atherogenic properties (12;13; 14). With regard to the role of adiponectin in the reproduction and its relationship with reproductive hormones, few studies are found in this field. So the aims of the present study were to determine the ability of clomiphene citrate to improve male reproductive hormones from bad effects resulted from atrazine exposure and the relation with adiponectin hormone.

Materials and Methods

The present study was conducted at Veterinary Medicine College – University of Basra .A total number of 30 adult albino male rats (*Rattus Rattus*) weighing 210 - 240 grams , and 10 – 13 weeks old were used in the current study. Animals were kept under normal temperature (22 - 28 °C) , and controlled lightening and provided with water and diet *ad libitum*. Animals were randomly divided into five equal groups each group consisted of 6 adult male rats as in the following :-

- 1-Control group : orally dosed with distilled water .
- 2-Group2 : orally dosed with atrazine 50 mg /kg B.W. daily for 30 days.
- 3-Group 3 : orally dosed with atrazine 50 mg /kg B.W. and 0.5 mg / kg B.W. clomiphene citrate daily for 30 days.
- 4-Group 4 : orally dosed with atrazine 50 mg /kg B.W. and 0.6 mg / kg B.W. clomiphene citrate daily for 30 days.
- 5-Group 5 : orally dosed with atrazine 50 mg /kg B.W. and 0.7 mg / kg B.W. clomiphene citrate daily for 30 days.

After experimental period (30 days) animals were sacrificed and blood were collected by direct heart puncture ,then blood samples were put in plain tube until coagulation , blood samples were centrifigated , serum was collected and distributed into many Eppendorf tubes, frozen at -20 C^o until used .

Hormonal assay

Testosterone, FSH, and LH hormones were assayed by using enzyme – linked immunosorbent assay (ELISA) kit manufactured by Human diagnostic company, Germany. Adiponectin hormone was assayed by using enzyme – linked immunosorbent assay (ELISA) kit manufactured by Assay Max company. USA.

Statistical analysis

Data were expressed as mean \pm SD. The comparisons between groups were performed with analysis of variance (ANOVA) by using computerized SPSS program (Statistical Program for Social Sciences). $P < 0.05$ was considered to be the lest limit of significance.

Results

It is clear, from table (1) that the dose of 50 mg / kg W.B. of atrazine caused a significant decrease ($p \leq 0.05$) in the concentration of testosterone , FSH, LH and adiponectin hormones , compared with the control group as well as when compared with all groups that received protective doses of clomiphene citrate 0.5 , 0.6 , 0.7 mg / kg B.W. . The group that received 0.5 mg /kg B.W. clomiphene citrate was still significantly

less in their hormonal values compared with control value.

Table (1):The protective role of clomiphene citrate against hormonal disturbances caused by atrazine exposure in male rats .

Parameters Groups	Testosterone ng/ml	FSH ng/ml	LH ng/ml	Adiponectin µg /ml
Control	4.94 ± 0.66 a	9.18 ± 1.16 a	3.18 ± 0.23 A	5.13 ± 0.63 a
Atrazine 50 mg/kg	1.92 ± 0.51 b	3.98 ± 0.66 b	1.52 ± 0.41 B	2.45 ± 0.55 c
Atrazine50mg/kg +clomid 0.5 mg/ kg	2.71 ± 0.46 b	5.17 ± 1.01 b	2.43 ± 0.53 C	3.44 ± 0.75 b
Atrazine50mg/kg +clomid 0.6 mg/kg	5.14 ± 0.68 a	9.00 ± 1.02 a	3.43 ± 0.49 A	4.57 ± 0.78 a
Atrazine50mg/kg +clomid 0.7mg/kg	5.42 ± 0.45 a	9.81 ± 1.43 a	3.82 ± 0.44 A	4.93 ± 0.76 a
LSD	2.23	3.83	0.63	0.99

N= 6

The numbers represent the mean ± Standard Deviation .

Different letters represent significant difference at ($p \leq 0.05$).

It is shown in table (1) that male rats which received 0.6 , 0.7 mg / kg B. W. clomiphene citrate as a protective treatment with atrazine exposure increased testosterone , FSH , LH and adiponectin hormones concentrations in their serum significantly ($p \leq 0.05$) compared with hormones of male rats exposed to atrazine.

Discussion

The significant decrease in the concentration of testosterone hormone in male rats treated with 50 mg / kg W.B. Atrazine might be attributed either to provoked degenerative changes in leydig cells which caused a declined in its steroidogenic capacity. This result is in line with previous observations (3 ;15) , or by disrupting hypothalamic-pituitary-testis axes through the decrease of LH secretion which lead to decrease testosterone secretion (16) These findings are consistent with the present study , so atrazine had affect testosterone secretion by direct leydig cells damage and indirect by reducing FSH and LH secretion (17) . According to Quignot *et al.* (18) atrazine reduce tesosterone concentration by induction of aromatase enzyme which leads to increase estrogen / testosterone ratio.

The protective treatment with all three doses of clmiphene citrate resulted in significant increase in serum testosterone, FSH and LH in male rats . According to the reproductive hormones results in treated male rats came in agreement with previous studies (19; 20). The elevation of the above hormones in present study was due to the administration of clomiphene citrate interfere with the normal negative feedback of sex steroids at hypothalamic and pituitary levels in order to increase endogenous gonadotropin-releasing hormone secretion from the hypothalamus and LH secretion directly from the pituitary. In turn, FSH and LH stimulate Leydig cells in the testis, and this lead to increased local testosterone production, thereby boosting spermatogenesis with a possible improvement in fertility. There may also be a direct effect of clomiphene citrate on testicular spermatogenesis or steroidogenesis (21).

According to Da Ros and Averbeck (22) 5 mg of clomiphene citrate for 6 months was very effective in improving testosterone level , and lowering cholesterol concentration in hypogonadal men.

The low levels of adiponectin hormone in male rats caused by atrazine exposure 50 mg /kg B.W. might be owing to elevated ROS levels in the endoplasmic reticulum (ER) and mitochondria that cause ER stress and mitochondrial stress in adipocytes (23) mitochondrial stress characterized by reduced intracellular ATP, mitochondrial membrane potential, and endogenous cellular respiration as well as an augmentation of oxidative stress (24) .

According to Koh *et al.* (25) the adiponectin in plasma and adipose tissue were significantly lowered when a reduction occurred in mitochondrial content and function in adipose tissue in mice .

The mitochondrial stress itself lead to ER stress by reduce ATP which supply the energy required by sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) to import Ca^{2+} into the lumen of the ER. This would cause Ca^{2+} depletion within the ER , which may trigger the ER stress response and impairment of protein folding and synthesis of

proteins because the ER plays a central role in protein folding and in quality control of newly synthesized proteins including protein hormones such as adiponectin (26).

The present study came in line with other study reported that elevation in endoplasmic reticulum stress-inducible factors such as Activating transcription factor 3 (ATF3) which is an endoplasmic reticulum stress-inducible factor in adipocytes and myocytes lead to a reduction in adiponectin level and adiponectin receptors expression (27).

Reduce ROS production may be a good therapeutic strategy for overcoming mitochondrial stress. In present study the clomiphene citrate in all three doses may exhibit antioxidant capacity (28;29) and decrease ROS production and reactivate mitochondrial function which relieve endoplasmic reticulum stress which led to increase protein folding and normalized adiponectin secretion (30).

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