PROTECTIVE ROLE OF CLOMIPHENE CITRATE FROM THE BIOCHEMICAL EFFECTS OF ATRAZINE EXPOSURE IN ADULT MALE RATS .

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(Received 22 October 2012,Accepted 3 December 2012)

Key words : Atrazine , clomiphene citrate, biochemical

ABSTRACT

The present study aimed to investigate the protective role of clomiphene citrate on biochemical effects which may result from atrazine exposure. Thirty adult male rats were used, divided randomly and equally into control group, atrazine 50 mg /kg group ,atrazine (ATZ) 50 mg /kg and different doses of clomiphene citrate(CC) 0.5 mg / kg, 0.6 mg / kg and 0.7 mg / kg daily for 30 days. The results showed that serum biochemical affected negatively by atrazine exposure. The atrazine exposure caused a significant elevation in serum total cholesterol , triglycerides ,LDL-C , VLDL-C ,AST , ALT , and creatinine in addition to decrease HDL-C . The CC treatment(0.6 and 0.7 mg / kg) seems to increase significantly HDL-C and reduced serum total cholesterol , triglyceride ,LDL-C and VLDL-C . Liver and kidney functions were improved by reducing serum AST , ALT and creatinine . The response to the dose of (0.5 mg / kg) of CC was fluctuating between having positive impacts by reducing significantly the AST , ALT or being ineffective in all lipids profile and serum creatinine.

INTRODUCTION

Atrazine is one of the most widely used agricultural pesticides all over the world, is now recognized to have disrupting effects on the endocrine systems of mammals(1-2). The hepatotoxicity of atrazine was investigated by studying clinical parameters related to hepatic function. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP)were

increased due to atrazine exposure. Atrazine administration resulted in increasing in the level of total serum lipids (3) . Atrazine induced oxidative stress in liver and kidney of mice, in terms of decreased activities of the various antioxidant enzymes, increased of lipid peroxidation and decreased content of reduced glutathione (4). In fish atrazine exposures resulted in slight ultrastructural changes in renal corpuscles, slight histopathological changes in the liver (5). Liver and kidney functions can improve by clomiphene citrate. Women with poly cystic ovarian syndrome (PCOS) can safely use clomiphene citrate renal function improved in patients with significant decreases in serum blood urea nitrogen levels , as well as creatinine. There were similar decreases in liver transaminases AST and ALT levels(6).Serum cholesterol reduced in women that treated with clomiphene citrate(7) .

In men with persistent hypogonadotropic hypogonadism clomiphene citrate treatment resulted in decrease in Serum triglycerides significantly (8).In rats serum cholesterol partially reduced with very low doses of clomiphene ctirate , but the reduction more pronounced with high doses of clomiphene citrate (9). Both isomers of clomiphene (zuclomiphene and enclomiphene) are effective in reducing serum cholesterol (10). In postmenopausal women clomiphene citrate treatment resulted in an increase in HDL cholesterol (11). There are no report about the protective role of clomiphene citrate from biochemical effects result from atrazine exposure. Therefore the aims of the present study to determine whether clomiphene citrate can protect from biochemical effects resulted from atrazine exposure in male rats.

MATERIAL AND METHODS

The present study was conducted at Veterinary Medicine College – University of Basrah . A total number of 30 adult albino male rats (Rattus Rattus) weighing 230 - 280 grams , and 10 – 12 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 : non treated .
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 an experimental period (30 days) animals were sacrificed. Blood samples were collected via cardiac puncture .

Biochemical Tests

The biochemical tests were conducted in Central Research Unit of Veterinary Medicine –Basrah university , by using a chemistry auto analyzer and Cholesterol, HDL-C , Triglyceride , AST , ALT , creatinine Liquicolor kits Manufactured by Human diagnostic company , Germany.

Statistical Analysis

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

RESULTS

Table (1) shows that the AST activity significantly increased ($p\leq0.05$) in male rats challenged with atrazine (50 mg/kg B.W) compared with control male rats , protective treatment with all doses of clomiphene citrate resulted in significant decrease ($p \leq 0.05$) in serum AST activity compared with atrazine exposed group , whereas the group treated with (0.5, 0.6 mg / kg B.W.) clomiphene citrate reduced the AST activity , but still higher significantly ($p \leq 0.05$) compared with control group , the treated group (0.7 mg / kg B.W.) was able to get the AST activity to its normal value compared with control one .

It is obvious that the ALT enzyme activity elevated significantly ($p \leq 0.05$) in the group that received atrazine (50 mg /kg B.W.) compared with the control group ,

protective treatment with (0.5, 0.6 and 0.7 mg /kg B.W.) clomiphene citrate reduced AST serum activity significantly than that in the group exposed to atrazine . The dose 0.7 mg / kg B.W. Was capable of getting the ALT activity to its normal value compared with the control group.The doses of 0.5 , 0.6 mg /kg B.W. clomiphene citrate still significantly higher than control values. There were no any significant differences between 0.5 and 0.6 mg /kg B.W. clomiphene citrate treated group when compared with each other.

Table (1)The protective role of clomiphene citrate from atrazine exposure on male rats some serum liver enzymes and serum createnine.(mean ± SD)

Parameters / Groups	AST IU/L	ALT IU/L	Createnine Mg /dl
Control	59± 6.56 d	28.0±2.89 c	0.25±0.042 b
Atrazine 50 mg/kg	95.83±4.75 a	41.5±3.08 a	0.42±0.053 a
Atrazine50mg/kg +clomid 0.5mg/kg	87.33±4.18 b	35.66±3.39 b	0.37±0.049 a
Atrazine50mg/kg +clomid 0.6mg/kg	70.83±5.12 c	34.33±3.38 B	0.26±0.035 b
Atrazine50mg/kg +clomid 0.7mg/kg	65.83±6.40 cd	29.50±3.39 C	0.27±0.036 b
LSD	8.5	4.8	0.098

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

Serum creatinine increased significantly ($p \leq 0.05$) with atrazine exposure compared with control group. The protective dose 0.5 mg / kg B.W. of clomiphene citrate decresed serum creatinine but still significantly higher than control group. The both protective doses 0.6 and 0.7 mg /kg B.W. reduce serum creatinine to a concentration almost similar to that concentration of the control group.

Table (2) indicated that the exposure to the atrazine (50 mg / kg B.W.) led to remarkable significant increase ($p \leq 0.05$) in serum total cholesterol compared with control group . The protective treatment of the animals with 0.6 and 0.7 mg /kg B.W. clomiphene citrate led to significant decrease ($p \leq 0.05$) in serum cholesterol compared with atrazine group.There were not significant difference ($p \geq 0.05$) between the two groups 0.6 and 0.7 mg / kg B.W. compared with control one , it seems that the dose (0.5

mg / kg B.W.) of the clomiphene citrate had not sufficient effect to cause a significant reduction in serum cholesterol after increased significantly ($p \leq 0.05$) due to atrazine.

It is clear that the atrazine 50 mg / kg B.W. had a significant increase ($p \leq 0.05$) in serum triglycerides whereas 0.6 and 0.7 mg / kg B.W. of clomiphene led to a significant decrease in serum triglycerided of those animals ,there were no significant difference between 0.6 and 0.7 mg / kg B.W. clomiphene citrate when compared with each other. The dose 0.5 mg / kg B.W. of clomiphene had not affected an elevation of lipid profile values compared with atrazine group .

Table (2)The protective role of clomiphene citrate from atrazine exposure on male rats serum lipids profile. .(mean ± SD)

Parameters / Groups	Total cholesterol mg / dL	TG mg / dL	HDL-C mg / dL	LDL-C mg / dL	VLDL-C mg / dL
Control	80.3±6.68 b	61.17 ± 6.97 C	46.67 ±4.97 A	18.21 ± 7.38 C	12.25 ± 1.39 b
Atrazine 50 mg/kg	95.8 ± 7.78 a	78.83 ± 2.14 A	36.0 ± 6.26 C	45.30 ± 4.65 A	15.20 ± 2.28 a
Atrazine50mg/kg +clomid 0.5mg/kg	93.17 ± 6.02 a	72.83 ± 6.70 Ab	36.50 ± 3.56 C	38.43 ± 3.66 A	14.40 ± 2.34 ab
Atrazine50mg/kg +clomid 0.6mg/kg	82.0 ± 6.29 b	70.17 ± 4.36 B	38.67 ± 3.39 Bc	28.47 ± 5.21 B	12.70 ± 1.33 b
Atrazine50mg/kg + clomid 0.7mg/kg	82.167 ± 4.36 b	70.0 ± 4.89 B	44.33 ± 5.47 Ab	18.22 ± 7.38 C	13.03 ± 1.67 ab
LSD	11	8.6	7.8	9.9	2.5

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

Atrazine exposure led to a significant ($p \leq 0.05$) reduction in the level of HDL-C in the blood serum of all groups compared with control group , except the protective dose of (0.7 mg /kg B.W.) clomiphene citrate were not significantly different compared with the control group . There were not any significant difference between 0.6 and 0.7 mg / kg B.W. clomiphene citrate when compared with each other.

It is obvious that serum LDL-C elevated significantly ($p \leq 0.05$) with atrazine exposure , protective treatment with (0.6 and 0.7 mg /kg B.W.) clomiphene citrate lower its serum level significantly compared with atrazine exposed group, even there were not any significant difference ($p \geq 0.05$) between the clomiphene 0.7 mg /kg B.W. treated group and control group.

It is seem that serum VLDL-C rose significantly ($p \leq 0.05$) in atrazine exposed group compared with control group . All groups that received clomiphene citrate as a protective treatment were not significantly different ($p \geq 0.05$) compared with control group , although male rats serum VLDL-C level in groups that received (0.5 and 0.7 mg /kg B.W.) clomiphene seems non significantly different from atrazine treated group .

DISCUSSION

The significantly higher AST and ALT activities in animals exposed to (50 mg / kg) atrazine compared with control groups (table 2) due to the leakage of aminotransferase (AT) enzymes from injured liver cells. This finding came compatible with other studies (3-12). On the contrary, another study reported that rats treated with 400 mg / kg atrazine for 14 consecutive days result in not significant elevation in serum ALT enzyme (13) .

ALT is thought to be more specific for hepatic injury because it is present mainly in the cytosol of the liver and in low concentrations elsewhere (14) . The elevation of ALT in the current study attributed specifically to the injury of liver cells caused by atrazine (12) , whereas the AST is a mitochondrial enzyme found in the heart, liver, skeletal muscle, and kidney and is normally present in plasma (15) . The elevated serum AST thought to be due to mitochondrial membrane disruption , elevation in intracellular Ca^{2+},

generation of reactive oxygen species (ROS) induced by atrazine which represent the cytotoxic mechanism caused by atrazine (16).

Treatment with all three doses of clomiphene citrate result in significant decrease in serum AST and ALT enzymes this results came in agreement with Aubuchon *et al.* (6) .

Atrazine exposure result in a significant increase in serum creatinine in male rats compared with control groups .Nephrotoxicity of atrazine is a consequence of its elimination through the kidneys (17) which leads to a decrease in creatinine clearance and proteinuria (3).

The exposure to atrazine (50 mg / kg) result in a significant elevation in serum total cholesterol , triglycerides , LDL-C , VLDL-C. With regard to the HDL it seems that atazine reduces the HDL-C significantly. This could result from down regulated steroidogenic activity from cholesterol which is a precursor for steroidogenesis resulting in a decrease of the end products and elevation of cholesterol (18) .

The increased serum LDL-C and VLDL-C could result from inhibition of scavenger receptor β1 by atrazine (18) . Scavenger receptor B1 inhibition result in elevation of LDL-C (19) whereas overexpression of the receptor have lower concentrations of VLDL-C and LDL-C (20- 21) .

Clomiphene citrate treatment in male and female rats resulted dose dependent improvement of serum lipids with regard to reduction of serum cholesterol , triglycerides , LDL-C , VLDL-C, as well as increase serum HDL-C . This result came in agreement with previous studies (7-9-11) . On contrary yasar and Ertuğrul (22) reported that clomiphene citrate may cause severe hypertriglyceridemia.

A dose-dependent decrease in serum cholesterol , triglycerides , LDL-C , VLDL-C may be attributed to the estrogenic biological activities of clomiphene citrate (23) .

الدور الوقائي لسترات الكلومفين من التأثيرات الكيموحيوية الناتجة من التعرض للاترازين في ذكور الجرذان البالغة

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الخلاصة

هدفت الدراسة الحالية الى اختبار الدور الوقائي لسترات الكلومفين من التأثيرات الكيموحيوية الناتجة من التعرض للاترازين. أستخدم ثلاثين من ذكور الجرذان البالغة قسمت عشوائيا وبالتساوي الى خمسة مجاميع ، مجموعة سيطرة ، مجموعة جرعت فمويا اترازين (50 ملغم / كغم) ، اترازين وجرع مختلفة من سترات الكلومفين (0.5 ملغم / كغم) ، (0.6 ملغم / كغم) ، و (0.7 ملغم / كغم) يوميا ولمدة 30 يوم. اظهرت النتائج بأن المعايير الكيموحيوية في المصل قد تأثرت سلباً بالتعرض للاترازين ظهر هذا من خلال ارتفاع الكوليستيرول ، الكلسيريدات الثلاثية ، البروتينات الدهنية المنخفضة الكثافة ، البروتينات الدهنية شديدة انخفاض الكثافة ، وانزيمات AST و ALT و الكرياتنين وانخفاض مستوى البروتينات الدهنية العالية الكثافة . اظهر العلاج بسترات الكلومفين (0.6 و 0.7 ملغم / كغم) زيادة معنوية في مستوى البروتينات الدهنية العالية الكثافة ، وانخفاض الكوليستيرول ، الكلسيريدات الثلاثية ، البروتينات الدهنية المنخفضة الكثافة ، البروتينات الدهنية شديدة انخفاض الكثافة ، وانزيمات AST و ALT و الكرياتنين . تذبذبت الاستجابة لجرعة 0.5 ملغم / كغم من سترات الكلومفين بين ان تكون مؤثرة ايجابيا بخفض انزيمات AST و ALT معنويا ، او ان تكون غير مؤثرة في التحليل الكلي للدهون والكرياتنين.

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