Biochemical and Histological Effects of Clomiphene Citrate on Liver of Female Albino Rat

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Abstract: The present work investigates the effects of the antiestrogen drug, clomiphene citrate (CC, clomid) on livers of albino rats. Many biochemical and histological changes were induced in these animals when treated with CC at doses of 10, 50 and 100 mg/kg body weight. Treating animals with doses of 50 and 100 mg/kg body weight caused elevations in serum cholesterol and triglyceride levels as well as significant changes in the total protein levels. Additionally, there were significant increases in the levels of liver function enzymes ALT, AST, ALP and fasting blood sugar in animals that were given clomiphene citrate. The histological changes in the livers of treated animals included cytoplasmic vacuolations in hepatocytes, leucocytic infiltrations, congestion of blood vessels and hyperplasia of bile ducts. In conclusion, biochemical analysis and histopathological alterations indicate that clomid causes hepatic damage in albino rats.

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1. Introduction

Polycystic ovary syndrome (PCOS) is a common cause of infertility and is often amenable to treatment with oral ovulation induction agents. For many years, clomiphene citrate was the first line of treatment for infertile women with polycystic ovary syndrome (Abu Hashim, 2012). Clomiphene citrate (CC), a selective oestrogen-receptor modulator, presumably works to induce ovulation by inhibiting negative, endogenous, oestrogen-feedback on the hypothalamic-pituitary axis, resulting in increased FSH secretion, follicular growth, and ovulation (Emily et al., 2010). However, clomiphene citrate has many adverse effects, such as ovarian enlargement, vasomotor flashes, nausea, vomiting, breast discomfort, headache, abnormal vaginal bleeding, visual symptoms, weight gain and shortness of breath. It has also been reported that CC induces acute pancreatitis (Siedentopf et al., 1997; Keskin et al., 2007), myocardial infarction (Duran and Raja, 2007), hypertriglyceridemia (Yasar and Ertugrul, 2009), deep vein thrombosis (Benshushan et al., 1995) and pulmonary embolism (Chamberlain and Cumming, 1986).

Two types of birth defects have been commonly reported to be associated with CC exposure in previous studies: neural tube defects (NTDs) and hypospadias. However, some results have confirmed that the association between CC and these birth defects was inconsistent (Greenland and Ackerman, 1995; Sorensen et al., 2005; Meijer et al., 2006; Wu et al., 2006). Reefhuis et al. (2011) have identified associations between the use of clomiphene citrate and anencephaly, Dandy Walker malformation, septal heart defects, muscular VSD, coarctation of the aorta, esophageal atresia, cloacal exstrophy, craniosynostosis and omphalocele. Clomiphene citrate has also been shown to cause ovarian and uterine abnormalities (Nagao and Yoshimura, 2001). Although it has been shown that CC enhances apoptotic processes in the ovaries, villi, and decidual tissues, CCeffect on apoptosis in the fallopian tube is still unknown (Shao et al., 2009). Rather, it has been suggested that CC might have adverse effects on the fallopian tube or on apoptotic signalling pathways in these tissues (Shao et al., 2009). The aim of the present work was to understand the possible effects of clomiphene citrate (clomid) on the livers of albino rats.

2. Materials and Methods

Adult female Sprague-Dawley rats with an average weight of 145-220 g were obtained from the animal house unit of the King Fahd Centre for Medical Research in Jeddah, Saudi Arabia. Ten animals per cage were placed in plastic cages (550x330x200 mm) at room temperature (22 ± 2 °C). The relative humidity was maintained at less than 40-65 %, and the animals were kept in alternating light and dark conditions for 12-hour periods. The rats were maintained on commercial food consisting of standard rat chow and had free access to drinking water. All animals received care in accordance with the methods approved under the institutional guidelines for the care and use of laboratory animals at the King Fahd Centre for Medical Research, Jeddah. Rat food pellets were purchased from the Saudi Grain Oils and Floor Mills organisation, Jeddah, Saudi Arabia. The sawdust bedding of the rat cages was changed three times a week and the cages were cleaned and sterilised.

Clomiphene citrate (Clomid)

Clomiphene citrate is an orally administered non-steroidal, ovulatory stimulant designated chemically as

2-[p-(2-chloro-1,2-diphenylvinyl) phenoxy] triethylamine citrate (1:1). A stock solution of clomiphene citrate was prepared by dissolving clomiphene citrate in distilled water; three concentrations of 10, 50 and 100 mg/ml (v/v) were prepared. Each concentration of clomid was given to the rats orally by intragastric intubation.

Experimental design

The animals used in this study were divided into four groups; each group consisted of 15 rats.

Group 1: The rats of this group were considered as controls. The animals in this group were dissected after two weeks, and the weight of each animal was measured before dissection.

Group 2: The rats of this group were fed daily with 10 mg of clomid/kg body weight for two weeks.

Group 3: The rats of this group were fed daily with 15 mg of clomid/kg body weight for two weeks.

Group 4: The rats of this group were fed daily with 100 mg of clomid/kg body weight for two weeks.

Histopathological examination

The treated animals and their controls were killed by cervical dislocation, quickly dissected and liver was removed, fixed in Bouin's fluid. After 24 h, tissues were rinsed three times in 70% ethanol, dehydrated using a graded ethanol series and then embedded in paraffin wax. Paraffin sections were cut into 5 micrometers thick slices and stained with haematoxylin and eosin and examined under light microscope.

Biochemical study

The rats were slaughtered using a sharp scalpel in the neck area, and the blood obtained was kept in small tubes coated with an anti-clotting reagent. The sera were obtained by centrifuging the blood samples and were stored at 20 °C for biochemical analysis. The biochemical analysis involved measuring levels of cholesterol, triglycerides, total protein, alkaline phosphatise (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT); the analysis was performed on a fully automated Hitachi 911 analyser (Tokyo, Japan). The Randox kits used for this analysis are commercially available and obtained from Randox Laboratories Ltd. (Ardmore, Crumlin, United Kingdom).

3. Results

Histological results

Fig. 1(A) shows the histology of the untreated albino rat liver (control). The hepatic parenchyma appears as regular cords arranged radically from the hepatic cells with eosinophilic, finely granular cytoplasm containing one or two prominent nuclei. Treating animals with clomid induces many histopathological alterations, as apparent in Fig. 1(B) from the livers of animals that were given a high dose of clomid (100 mg/kg body weight). Fig. 1(B) shows that the hepatic veins became enlarged and congested. In treated animals, the sinusoidal spaces appeared narrowed and filled with blood, and the Kupffer cells were overactivated, as shown in Fig. 2(A). Additionally, hyperplasia of the bile ducts was observed, as shown in Fig. 2(B). Additionally, in treated animals, masses of leucocytic infiltrative cells were abundant in many areas of the liver tissue (Fig. 3(A)), and the hepatocytes appeared to have cytoplasmic vacuolations with pyknotic nuclei (Fig. 3(B)).

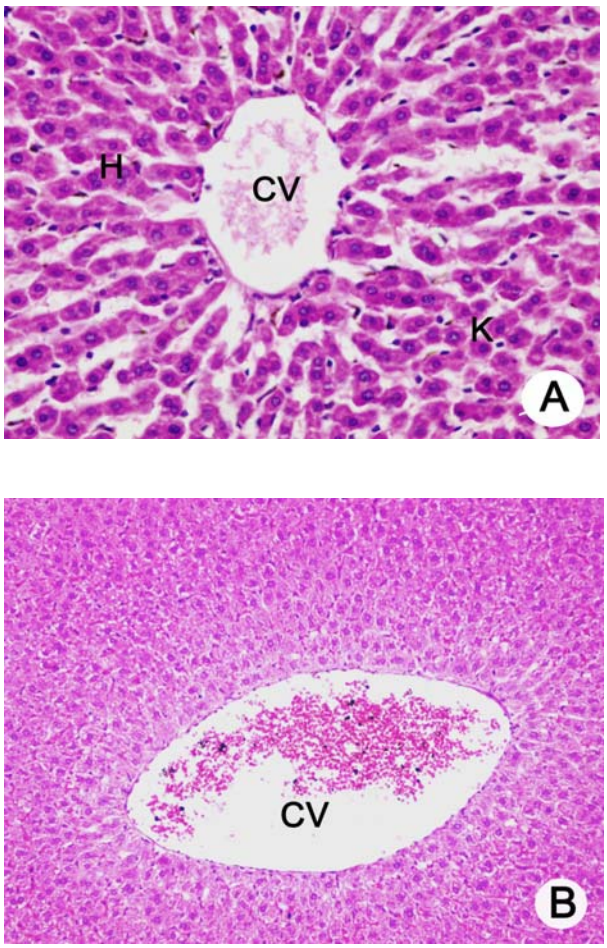

Figure 1. (A) Histology of the untreated albino rat liver (control), X 400, **(B)** Enlarged and congested central vein of liver of clomid-treated rat, X 250

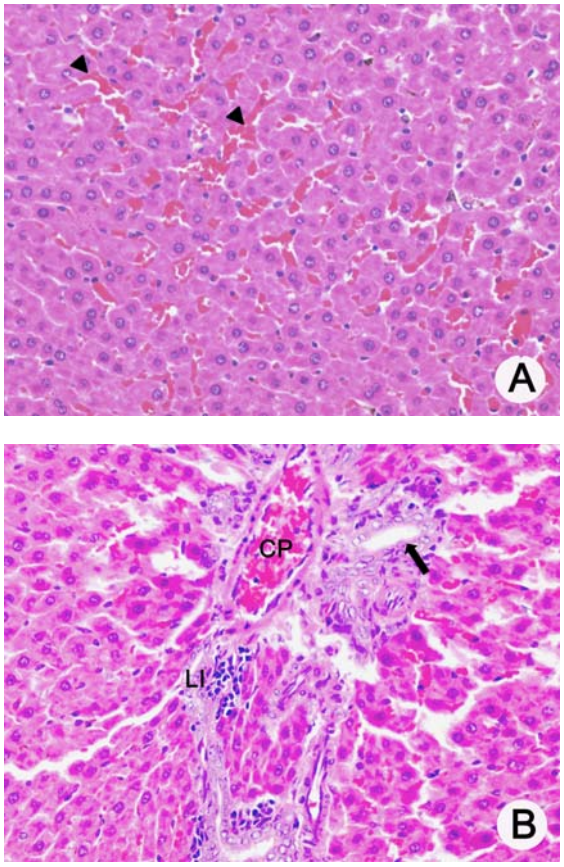

Figure 2. (A) Sinusoidal spaces appear narrowed and filled with blood (arrow head), and the Kupffer cells are overactivated. **(B)** Hyperplasia of the bile duct (arrow), leucocytic infiltrations (LI), congested portal vein (CP), X 400.

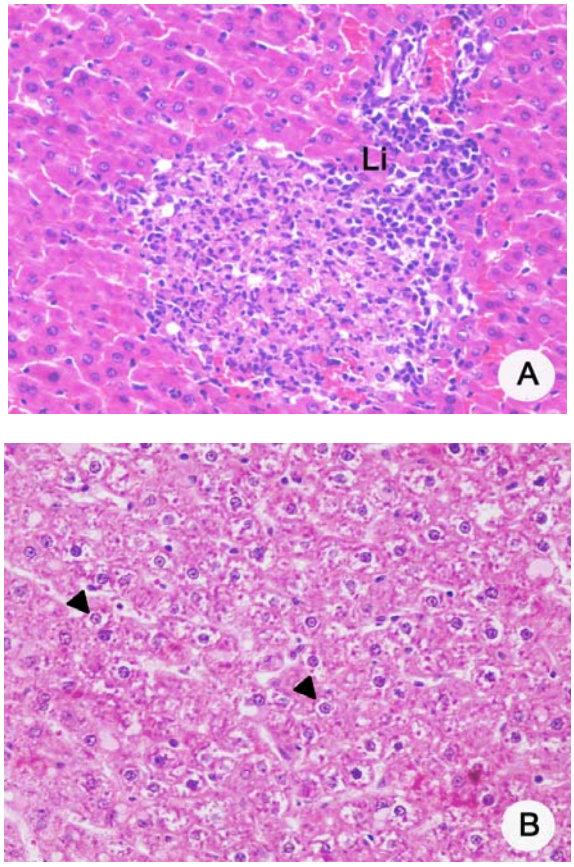

Figure 3. (A) Mass of leucocytic infiltrative cells (Li), **(B)** Hepatocytes with cytoplasmic vacuolations (arrow head) with pyknotic nuclei ,X 400.

Biochemical results

Data in Figures 4 and 5 show that there is a significant increase in the cholesterol and triglyceride levels in animals that were given clomid at a dosage 100 mg/kg body weight. In contrast, animals given clomid at doses of 50 and 100 mg/kg body weight showed a significant decrease in the total protein levels. The mean values for the total protein concentrations were 71.2 ± 2.5, 60.8 ± 1.5 and 54.6 ± 2.9 for the control, 50 mg/kg body weight and 100 mg/kg body weight groups, respectively (Fig. 6). Figure 7 shows a significant increase in ALP levels in animals that were given clomid at doses of 50 mg/kg body weight and 100 mg/kg body weight. The mean values for the ALP levels were 123.8 ± 5.8, 148.2 ± 8.9 and 152.0 ± 6.7 for the control, 50 mg/kg body weight and 100 mg/kg body weight groups, respectively. Similarly, there were significant increases in the AST and ALT levels in animals that were treated with clomid at doses of 50 mg/kg body weight and 100 mg/kg body weight. The mean values of AST levels were 118 ± 7.9, 179.4 ± 5.33 and 182.4 ± 7.5 for the control, 50 mg/kg body weight and 100 mg/kg body weight groups, respectively (Fig. 8). The mean values of ALT levels were 58.6 ± 3.4, 72 ± 9.9 and 88.4 ± 7.95 for the control, 50 mg/kg body weight and 100 mg/kg body weight groups, respectively (Fig. 9).

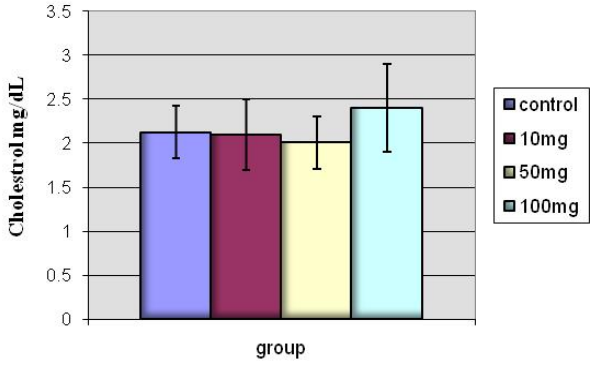

Figure 4: Cholesterol levels in the different animal groups.

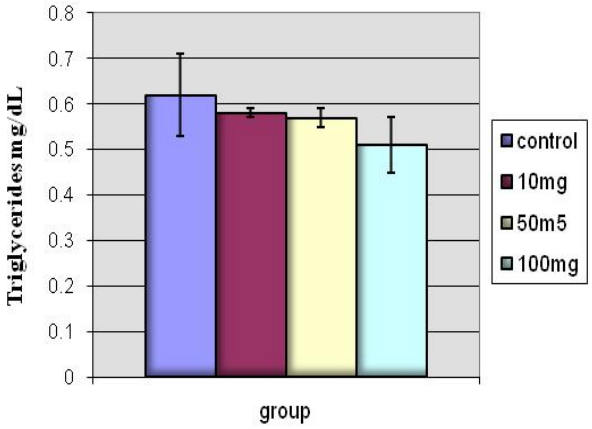

Figure 5: Triglyceride levels in the different animal groups.

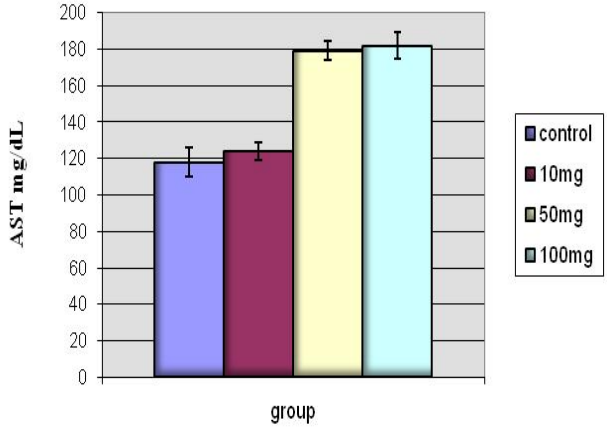

Figure 8: AST levels in the different animal groups.

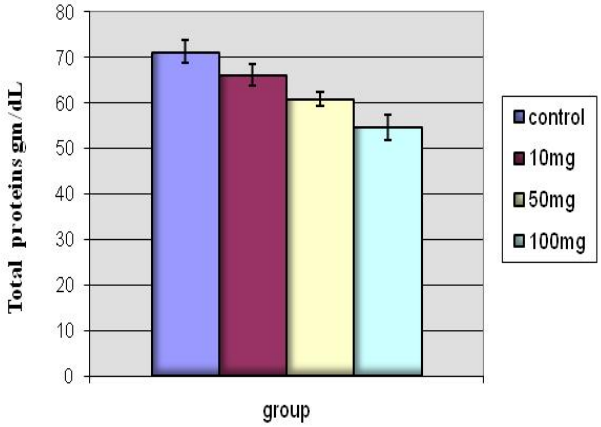

Figure 6: Total protein levels in the different animal groups.

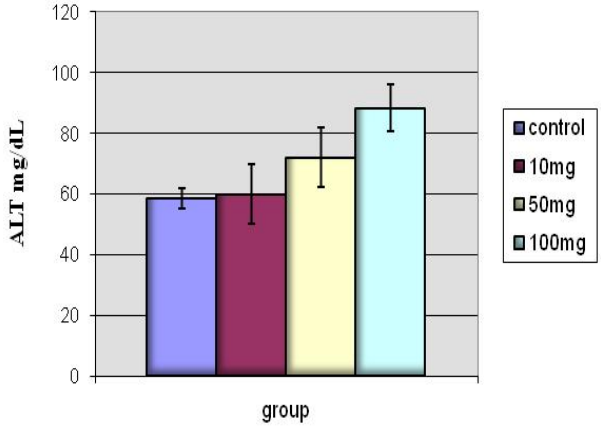

Figure 9: ALT levels in the different animal groups.

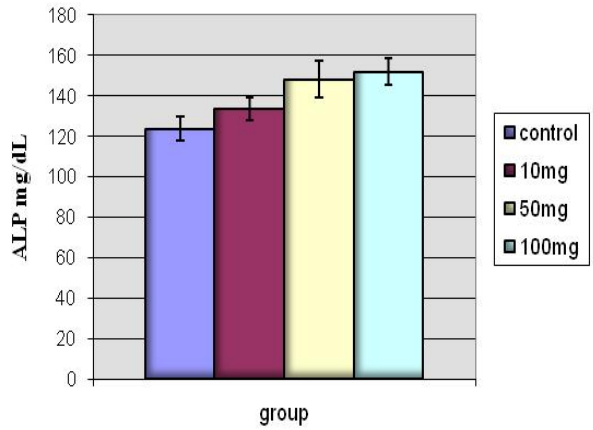

Figure 7: ALP levels in the different animal groups.

4. Discussion

Histopathological evaluation of livers of the clomid-treated animals showed remarkable and pronounced alterations when high doses of clomid were given. It is noteworthy that treated livers appeared to have congestion of veins, leucocytic infiltrations and cytoplasmic vacuolations of the hepatocytes. Shimono et al. (1998) have reported that women who took CC suffered from ovarian hyperstimulation syndrome complicated by hepatic injury. Ghia and Mereto (1989) have reported that gamma-glutamyltranspeptidase-positive foci were induced in livers of female rats that were treated with clomiphene citrate. Focal nodular hyperplasia of the liver was observed after clomiphene citrate treatment in a young boy (Morocz et al., 1986). Cytoplasmic vacuolations were observed in hepatocytes of clomid-treated animals and are considered a form of cell injury, which is the most frequent in parenchymal cells of the liver with wide networks of internal membranes

responsible for ions pumping (Mori, 1983). Cytoplasmic vacuoles develop due to the accumulation of ions and water in the cytosol and rapidly pass through leaky membranes of cell organelles. Massive accumulation of fluids in the vacuoles finally leads to cell lysis (Gores et al., 1990).

The results from the present study show that clomid causes significant elevation in cholesterol and triglyceride levels. Similarly, Yasar and Ertugrul (2009) reported that a woman who had a history of polycystic ovary syndrome was given clomiphene citrate (CC) for induction of ovulation and showed severe hypertriglyceridemia. Chaudhuri et al. (1990) reported significant increases in lipid and lipoprotein levels, and minor changes in liver function enzymes and fasting blood sugar in women who were given clomiphene citrate. It has been speculated that treating rats with clomid increases tissue lipogenesis and is probably achieved by increasing the concentrations of acetyl CoA, the precursor of cholesterol biosynthesis (Siedentopf et al., 1997; Haschek and Rousseauk, 1998). The total protein levels decreased in the sera of clomid-treated rats. In this direction, Su et al. (2002) have reported that the antiestrogens (tamoxifen, clomiphene and nafoxidine) were found to inhibit the phospholipid/Ca^{2+}-dependent protein kinase. This reduction in protein content may be attributed partially to the decreased levels of hepatic protein synthesis in cells that have suffered pathological changes due to hyperactivity of the hydrolytic enzymes (Ganong, 1997).

Alkaline phosphatase (ALP), AST and ALT levels increased significantly in rats that were given high doses of clomid. This finding is in agreement with results of Shimono et al. (1998) who observed elevated levels of transaminases (AST, ALT) in women treated with clomiphene citrate for a month. Transaminase levels are considered to be sensitive parameters in evaluating liver function and damage (Sherlock, 1981). Hatoff and Hardison (1980) reported that increases in serum levels of these enzymes were primarily attributed to either acute hepatocellular damage or extrahepatic obstruction or both. Thus, increases in serum ALT and AST levels, together with histopathological alterations, indicate that clomid causes hepatic damage in albino rats.

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