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Fluconazole, a Fungal Cytochrome P450 Enzyme Inhibitor: The Potential Role in Augmenting Hepatotoxicity and Hyperinsulinemia Induced by Dexamethasone in Rats

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INSULIN resistance (IR) is a widespread health problem characterized by failure of cell responses to insulin. IR increases the risk of fungal infections. Long-term administration of dexamethasone is a therapeutic need accompanied IR and fungal infections. Fluconazole is the first-line antifungal therapy that inhibit fungal cytochrome P450 (CYP450) enzyme. It has a hepatotoxic effect and moderate inhibitory effect on multiple CYP450enzymes that could affect the pharmacokinetics of drugs used to treat insulin resistance such as metformin. This study aims to evaluate the effect of administration of fluconazole on liver function, CYP450concentration and level of insulin resistance induced by dexamethasone and high fat diet. Insulin resistance in rats was induced by dexamethasone (0.1mg/kg/day s.c) (DEX) for 2 weeks, after eight weeks of high fat diet (HFD) until the end of the experiment. Oral administration of fluconazole (20, 40 mg/kg) (FLU20, 40) was started concomitantly with DEX for 2 weeks. Metformin (400mg/kg) (MET) was used as a reference treatment for IR and administered orally for 2 weeks with DEX and FLU (20, 40). Concomitant administration of FLU either 20mg/kg or 40 mg/kg with DEX showed significant increase in IR, AST and ALT compared by that caused by DEX alone or with MET. FLU showed a significant decrease in serum CYP450 that elevated by DEX. Massive fibrosis was noticed in histological assessments of liver tissues of HFD+DEX+FLU (20, 40) groups. In conclusion administration of FLU in case of IR induced by HFD+DEX resulted in deterioration of liver functions and decrease in the therapeutic efficacy of MET.

Keywords: Dexamethasone, Fluconazole, Insulin Resistance, Metformin, Cytochrome P450.

Introduction

Insulin resistance (IR) is a widespread health problem;[1] it is characterized by hyperinsulinemia and inability of insulin to increase glucose uptake and utilization in the skeletal muscle and fat tissues [2]. Uncontrolled IR is accompanied by many medical complications such as fungal infections[3]. Dexamethasone is one of the most commonly prescribed anti-inflammatory corticosteroid to patients with rheumatologic syndromes, asthma, vision-threatening eye disease, skin disorders, glomerulopathies, pain syndromes, organ transplants, malignancies and other ailments [4,5].

Despite the therapeutic necessity of using dexamethasone, it has many potential side effects.IR is one of the possible results of long term administration of DEXdue to the effect of DEX in potentiating lipolysis and

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decreasing glucose oxidation and uptake[5-8]. DEX increases the risk of fungal infections by modulating of T-cells proliferation [9]. DEX stimulates the activity of some CYP450 isoforms and this affect the pharmacokinetics of drugs used in treatment of diabetes and IR such as metformin [10,11].

Fluconazole is a first generation triazole antifungal medication and it is considered as the first-line antifungal therapy in many cases of immune compromised people including transplant patients [12]. It acts by inhibition of the fungal CYP450 enzyme 14α-demethylase and therefore prevents the conversion of lanosterol to ergosterol, which is the essential component of the fungal cytoplasmic membrane. Fluconazole is routinely prescribed to control vulvovaginal candidiasis, and cutaneous candidiasis in diabetic patients [13,14]. Despite fluconazole is considered as a safe antifungal drug due to its selective inhibition of fungal CYP45, its hepatotoxic effect and inhibitory effect on some mammalian CYP450 isoforms, the proteins which able to catalyze various enzymatic reactions including the metabolism of xenobiotics, were also recorded as a drawback of fluconazole[15-17].

Studies that investigated the concurrent administration of DEX and FLU were more concerned about their pharmacokinetics and their synergetic antifungal effect against resistant Candida albicans [18,19].

From previous literatures, it is concluded that combined administration of fluconazole and dexamethasone induces many side effects.This study aims to evaluate the effect of fluconazole on serum CYP450, hepatotoxicity and insulin resistance that induced by dexamethasone in rats.

Material and Methods

Animals

Adult male albino Wistar rats, weighing 200-230 g, were used. They were obtained from the animal house colony of the National Research Centre (Dokki, Giza, Egypt) and were housed in plastic cages covered with wood shavings flooring. Animals were kept for at least one week in the laboratory room prior to testing under standard housing conditions (room temperature 25ºC with alternating 12 hours light and dark cycles) and were allowed free access to water and food ad libitum. They were fed a standard rat pellet diet for normal group and fat-rich diet for other rats. All animals procedures were performed in accordance to the Institutional Ethics Committee of the National Research Centre (PTNo.12127).

Drugs

Dexamethasone: (Dexamethasone®) sodium phosphate ampoules were purchased from Amriyafor Pharmaceutical Industries, Alexandria-Egypt.

Fluconazole: (Diflucan®): purchased from Pfizer Company.

Metformin: (Cidophage®): purchased from Cid Company

Chemicals and test reagent kits

Rat insulin ELISA kit was obtained from Glory Science Co., Ltd.

Rat Cytochrome P450 1A2 (CYP 1A2) ELISA kit was obtained from Cloud-Clone Corp.

Rat aminotransferase (AST) colorimetric kit was obtained from Biodiagnostic Co

Rat alanine amino transferase (ALT) colorimetric kit was obtained fromBiodiagnosticCo

Experimental design and treatment protocol

Animals were allowed free access to HFD ad libitum for 8 weeks except for normal rats. All groups except the normal control and the high fat diet (HFD) control groups were injected dexamethasone (0.1mg/kg/days.c) (DEX) for two weeks starting after 8 weeks of HFD application or regimen. Dose, duration and route of administration of DEX to induce insulin resistance were chosen after pilot studies and according to Okumura et al., and Caro and Amatruda[19,20]. HFD were prepared without fructose according to method described by Basciano et al.,[21]. All treatments were administered for two weeks after eight weeks of administration of HFD that continued to the end of experiment. Metformin (MET) was selected as a reference drug used to treat IR; it was administered orally in a dose of 400mg/kg/day [22]. Doses of fluconazole (20,40mg/kg/day) (FLU 20, 40) were selected and administered orally in accordance with Fisher et al., [23].

Sixty four rats were randomly assigned into eight groups; as follows:

Normal control, HFD control group, (HFD+DEX) group, (HFD+DEX+MET) group, (HFD+DEX+FLU20) group, (HFD+ DEX+FLU4) group, (HFD+DEX+FLU40)

group, (HFD+ DEX+ FLU20+MET) group and (HFD+DEX+FLU40+MET) group.

Glucose tolerance test (GTT)

Glucose tolerance test (GTT) was recorded at the end of the experiment on overnight fasted rats that injected with glucose i.p. Blood samples were collected before injection of glucose and fasting blood glucose (FBG) were measured. Blood drops were collected from the tails of rats to measure blood glucose 30, 60, 90 and 120 min after injection using Ok meter® glucose testing strips to be analyzed by the commercially available glucometer (OK meter, Biotech Co., Ltd., Taiwan) [24].

Blood biochemical parameters

Blood samples were withdrawn via the retro-orbital plexus of all rats at the end of the experiment. Rats were fasted for at least 12 hr before blood collections. Blood samples were centrifuged at 3000 rpm. Serum was separated for measurement of insulin.

Determination of insulin resistance

Insulin resistance (IR) was calculated using homeostasis model assessment of (HOMA- IR) [25].

IR= fasting blood glucose (FBG) mmol/l X serum insulin **(**μIU/ml) /22.5

Histological assessment studies

Liver samples of all animals were dissected immediately after death. The specimens were then fixed in 10 % neutral-buffered formalin saline for 72 hours at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6μm thick were cut and stained with haematoxylin and eosin for histologicalinvestigation[26]. Images were examined and photographed under a digital camera (Microscope Digital Camera DP70, Tokyo, and processed using Adobe Photoshop version 8.0).

Statistical analysis

Data were expressed as mean ± SE of the mean of 8 experiment. Statistical analysis were carried out using one-way ANOVA, followed by Tukey's multiple comparison tests atp<0.5.

Results

Results of glucose tolerance test (GTT) for allgroups

Blood glucose (mg/dl) level was increased in all dexamethasone treated groups in comparison with normal and HFD control groups. There was a significant elevation in GTT in the HDF+ DEX treated groupby (18%, 144% and 196% at 30, 60 and 120 minrespectively), (12%, 102% and 114% at 30,60 and 120 minre spectively) for HDF+ DEX+ METtreatedgroup,(41%,360% and 291% at 30, 60 and 120min respectively) for HDF+DEX+FLU20, (36%, 367%and316% at 30, 60 and 120 min respectively) for HDF+DEX+ FLU40, (19%, 268% and 288% at30,60 and 120min respectively) for HDF+DEX+ MET+FLU20, (26%, 300% and 309% at 30, 60 and 120 min respectively) for HDF+DEF+ MET+ FLU40 at the end of 10th week in comparison with normal and control groups as shown in Fig. 1.

Results of insulin resistance and serum AST, ALT andCYP450

HDF+DEX treated group

Rats that treated with HDF+DEX showed a significant increase in serum insulin, HOMA-IR, AST, ALT and CYP450 by approximately 100%, 119%, 44%, 140% and 104% respectively in comparison with normal and control groups as shown Fig. 2, 3, 4, 5 and 6, respectively.

HDF+DEX+MET treated group

Rats that treated with HFD + DEX + MET showed insignificant differences in the result of AST and a significant increase in serum insulin, HOMA-IR, ALT and CYP450 by approximately 30%, 39%, 45% and 44% respectively in comparison with normal and control groups but they were significantly less than the findings in the rats that treated with only HDF+DEX as shown Fig. 2, 3, 4, 5 and 6 respectively.

HDF+DEX+ FLU 20 treated group

Rats that treated with HFD+DEX+FLU20 showed a significant increase in serum insulin, HOMA-IR AST, ALT and CYP450 by approximately 90%, 141%, 296%, 455% and 47% respectively in comparison with normal and control groups. Results of ALT and AST were significantly higher than those of groups that treated with HDF + DEX or HFD + DEX + MET. The concentration level of CYP450 was lowered significantly if compared with HDF + DEX group as shown Fig. 2, 3, 4, 5 and 6, respectively.

HDF+DEX+ FLU 40 treated group

Rats that treated with HFD+DEX+FLU40 showed a significant increase in serum insulin, HOMA-IR, AST, ALT and CYP450 by approximately 130%, 197%, 322%, 487% and 61% respectively in comparison with normal and control groups. Results of insulin, ALT and AST were significantly higher than those of groups that

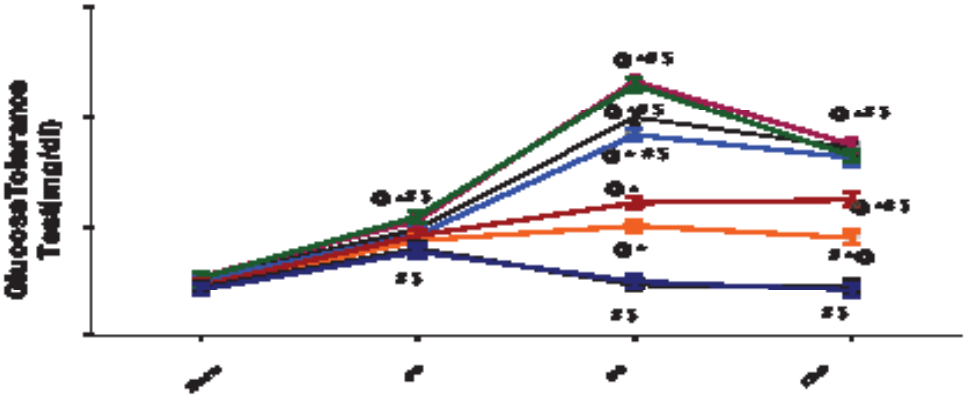

Fig. 1. Effect of high fat diet, dexamethasone (0.1mg/kg), metformin (400mg/kg) and fluconazole (20, 40 mg/kg) on glucose tolerance test (mg/dl) after 10thweek.

Insulin resistance in rats was induced by subcutaneous injection of dexamethasone for two weeks, after eight weeks of feeding freely them HFD and continued until the end of the experiment.

Data were expressed as mean ± SEM (n = 8). The statistical comparison of difference between groups was carried out using one-way ANOVA followed by Tukey's multiple comparison tests.
At $p< 0.05$

@ Significantly different from normal group
*Significantly different from HFD control group
Significantly different from DEX group
$ Significantly different from MET group

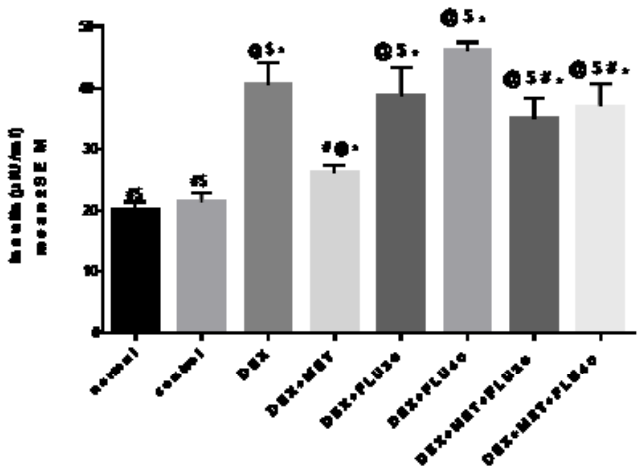

Fig. 2. Effect of high fat diet, dexamethasone (0.1mg/kg), metformin (400mg/kg) and fluconazole (20, 40 mg/kg) on serum insulin (µIU/ml).

Insulin resistance in rats was induced by subcutaneous injection of dexamethasone for two weeks, after eight weeks of feeding freely them HFD and continued until the end of the experiment.

Data are expressed as mean ± SEM (n = 8). The statistical comparison of difference between groups was carried out using one-way ANOVA followed by Tukey's multiple comparison tests.
At $p< 0.05$

@ Significantly different from normal group
*Significantly different from HFD control group
Significantly different from DEX group
$ Significantly different from MET group

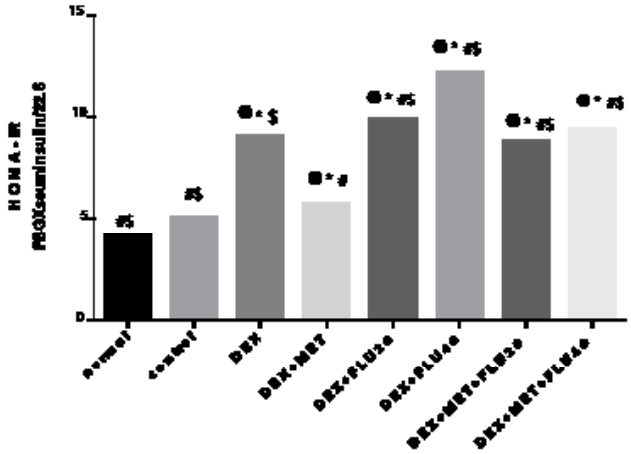

Fig. 3. Effect of high fat diet, dexamethasone (0.1mg/kg), metformin (400mg/kg) and fluconazole (20, 40 mg/kg) on insulin resistance using homeostasis model assessment (HOMA- IR).

Insulin resistance in rats was induced by subcutaneous injection of dexamethasone for two weeks, after eight weeks of feeding freely them HFD continued until the end of the experiment.

Data are expressed as mean (n =8). The statistical comparison of difference between groups was carried out using one-way ANOVA followed by Tukey's multiple comparison tests.
At $p< 0.05$

@ Significantly different from normal group
*Significantly different from HFD control group
Significantly different from DEX group
$ Significantly different from MET group

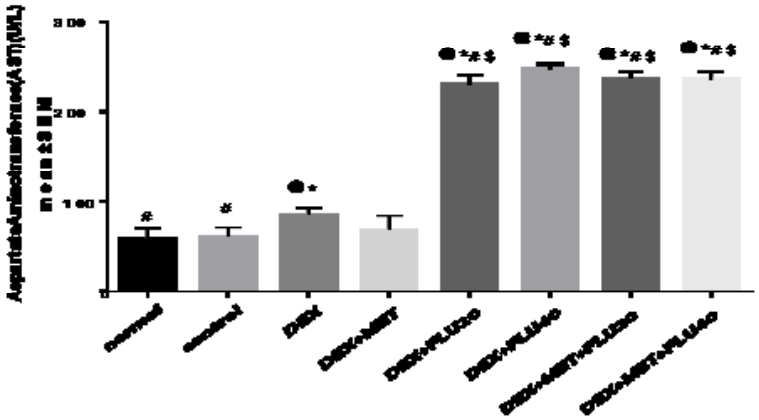

Fig. 4. Effect of high fat diet, dexamethasone (0.1mg/kg), metformin (400mg/kg) and fluconazole (20, 40 mg/kg) on serum liver aspartate aminotransferase (AST) (U/L).

Insulin resistance in rats was induced by subcutaneous injection of dexamethasone for two weeks, after eight weeks of feeding freely them HFD continued until the end of the experiment.

Data are expressed as mean ± SEM (n = 8). The statistical comparison of difference between groups was carried out using one-way ANOVA followed by Tukey's multiple comparison tests.
At $p< 0.05$

@ Significantly different from normal group
*Significantly different from HFD control group
Significantly different from DEX group
$ Significantly different from MET group

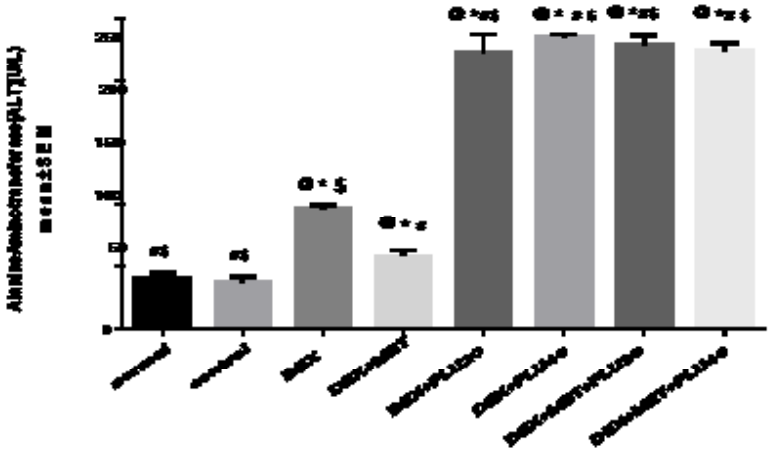

Fig. 5. Effect of high fat diet, dexamethasone (0.1mg/kg), metformin (400mg/kg) and fluconazole (20, 40 mg/kg) on serum liver alanine transferase (ALT) (U/L).

Insulin resistance in rats was induced by subcutaneous injection of dexamethasone for two weeks, after eight weeks of feeding freely them HFD continued until the end of the experiment.

Data are expressed as mean ± SEM (n = 8). The statistical comparison of difference between groups was carried out using one-way ANOVA followed by Tukey's multiple comparison tests.
At $p< 0.05$

@ Significantly different from normal group
*Significantly different from HFD control group
Significantly different from DEX group
$ Significantly different from MET group

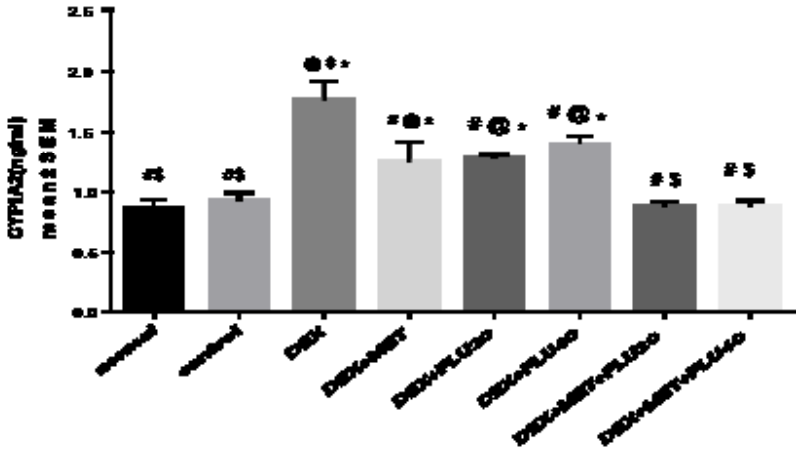

Fig. 6. Effect of high fat diet, dexamethasone (0.1mg/kg), metformin (400mg/kg) and fluconazole (20, 40 mg/kg) on serum Cytochrom p450 (cyp 1A2).

Insulin resistance in rats was induced by subcutaneous injection of dexamethasone for two weeks, after eight weeks of feeding freely them HFD continued until the end of the experiment.

Data are expressed as mean ± SEM (n = 8). The statistical comparison of difference between groups was carried out using one-way ANOVA followed by Tukey's multiple comparison tests.
At $p< 0.05$

@ Significantly different from normal group
*Significantly different from HFD control group
Significantly different from DEX group
$ Significantly different from MET group

treated with HDF+DEX or HFD+DEX+MET. Concentration of CYP450 was lowered pronouncedly if compared with HDF+DEX group as shown Fig. 2, 3, 4, 5 and 6, respectively.

HDF+DEX+ FLU 20+MET treated group

Rats that treated with HFD+DEX+ FLU20+ MET showed a significant increase in serum insulin, HOMA-IR, AST, ALT by approximately 75%, 114%, 306%, 472% respectively in comparison with normal and control groups. Results of ALT and AST were significantly higher than those of groups that treated with HDF+ DEX or HFD + DEX + MET. Serum insulin was decreased significantly when compared with HDF+DEX group. Concentration of CYP450 gave insignificant difference in comparison with normal and control groups as shown Fig. 2, 3, 4, 5 and 6 respectively.

HDF+DEX+ FLU 40+MET treatedgroup

Rats that treated with HFD+DEX+FLU40+ MET showed a significant increase in serum insulin, HOMA-IR, AST, ALT by approximately 85%, 129%, 303%, 460% respectively in comparison with normal and control groups. Results of ALT and AST were significantly higher than those of groups that treated with HDF+DEX or HFD+DEX+MET. Serum insulin was decreased significantly when compared with HDF+DEX group. Concentration of CYP450 gave insignificant difference in comparison with normal and control groups as shown Fig. 2, 3, 4, 5 and 6 respectively.

Histological assessment

A photomicrograph of a liver tissue section from normal control rats showed normal hepatocytes arranged in cords radiating from the central vein as shown in Fig. 7a.

A photomicrograph of a liver tissue section from HFD control rats showed normal hepatocytes with large vesicular nuclei but with noticeable blood sinusoids' dilatation as shown 7b.

A photomicrograph of a liver tissue section from rats treated with HDF+DEX showed severe dilatation and congestion of main blood vessels as shown in Fig. 7c.

A photomicrograph of a liver tissue section from rats treated with HDF+DEX+MET showed normal hepatocytes with no dilated blood sinusoids in between, but with mild thickening of central vein's wall and fibrosis around as shown in Fig. 7d.

A photomicrograph of a liver tissue section from HDF+DEX+FLU groups showed massive fibrosis and markedly dilated central vein with vacuolar degeneration (and pyknotic nuclei as shown in Fig. 7e.

A photomicrograph of a liver tissue section from rats treated with HDF+DEXMET+FLU (20mg and 40mg) showed many hepatocytes with vacuolar degeneration (black arrow). mild dilatation and congestion of blood sinusoids as shown in Fig. 7f.

Discussion

In the present study normal diet was replaced by HFD was used instead to potentiate the effect of dexamethasone as an inducer of insulin resistance in rats. It was reported by Stojanovska et al., that administration of HFD and dexamethasone resulted in higher insulin resistance in rats compared to those treated with dexamethasone alone [22]. This finding could be correlated to the role of HFD in the stimulating of the expression of several proinflammatory cytokines and inflammatory responsive proteins in hypothalamus and this leads to impairment of the insulin- signaling pathway as described by Prada et al., [23].

The current data revealed that dexamethasone administration resulted in a significant increase in insulin resistance when compared with normal and the HFD control groups. These results were in accordance with Stojanovska et al., and Okumura et al., and Buren et al.,[5,19,27]. This could be explained by the ability of dexamethasone to prevent glucose uptake by reducing the net translocation of the glucose transporter GLUT4 as pointed in Raymond R. Russell et al.,[28]**.** Administration of metformin to the rats resulted in a significant decrease in insulin resistance and showed obvious protective effect for liver tissues when compared with the groups that treated with HDF+DEX+FLU (20, 40). These findings were in line with those of Thomas et al., and Li et al., [29,30]. These results could be explained by the stimulating effect of metformin for AMP-activated protein kinase (AMPK) activity in both liver and skeletal muscle and by its downstream effect on acetyl-CoA carboxylase (ACC) [31,32].

In the present study, concomitant administration of fluconazole with dexamethasone caused further more increase in insulin resistance when compared with the effect of dexamethasone alone. These results may be explained by the negative impact of fluconazole on liver functions that noticed in this study as altered liver hyperinsulinemia could be resulted due to hepatic parenchymal cell damage or due to portal-systemic

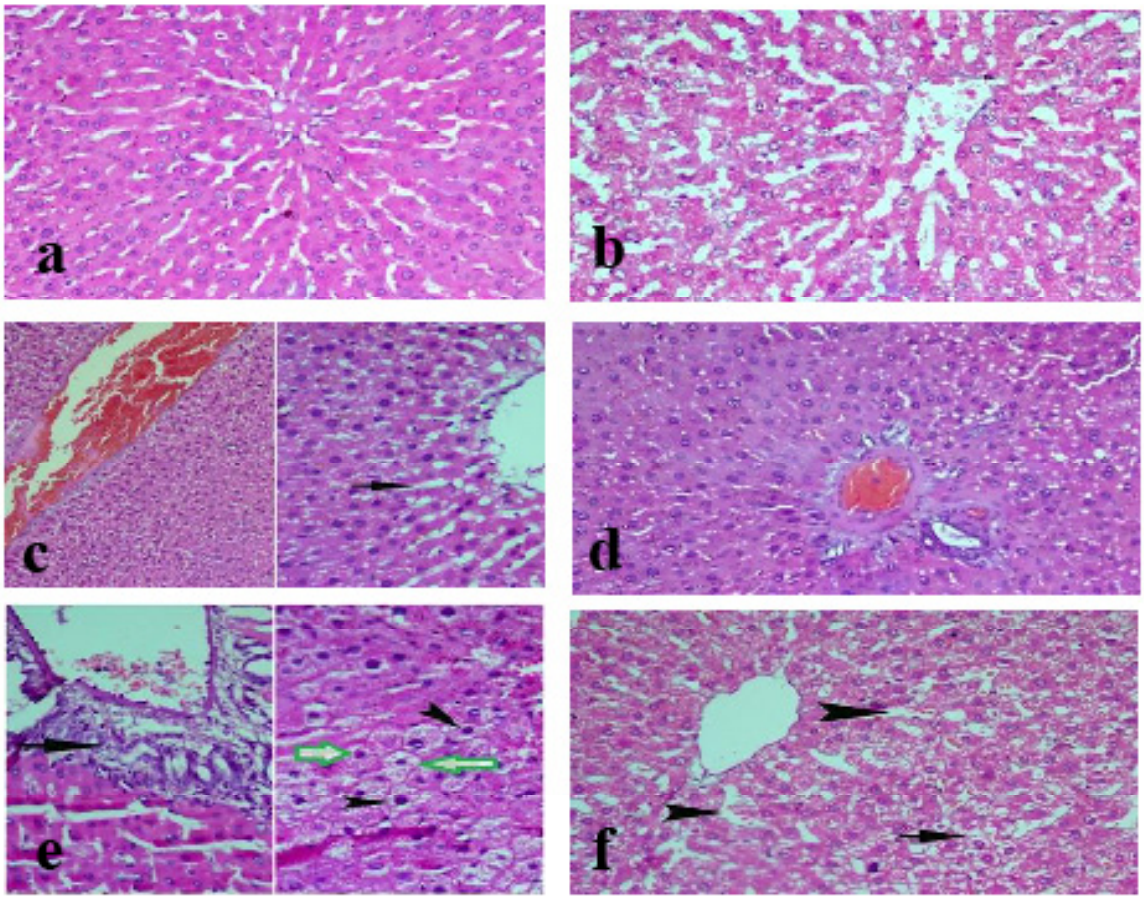

Fig. 7. A photomicrograph of a liver tissue sections from rats in all groups (Hx & EX 200): (a); section from a normal rats showed normal hepatocytes arranged in cords radiating from the central vein, (b); section from a control HFD rats showed normal hepatocytes with large vesicular nuclei but with noticeable blood sinusoids' dilatation, (c); section from a rats treated with HDF + DEX showed severe dilatation and congestion of main blood vessels on the left side of figure, while the right side of figure shows mild dilatation of blood sinusoids (black arrow) and some hepatocytes with pyknotic nuclei, (d); section from a rats treated with HDF + DEX + MET showed normal hepatocytes with no dilated blood sinusoids in between, but with mild thickening of central vein's wall and fibrosis around (e); section from HDF + DEX + FLU groups showed on the left side of the figure massive fibrosis (arrow heads) around the markedly dilated central vein. On the right side of figure hepatocytes with vacuolar degeneration (green arrow) and pyknotic nuclei (black arrow) were observed, A photomicrograph of a liver tissue section from HDF + DEX + FLU groups showed on the left side of the figure massive fibrosis (arrow heads) around the markedly dilated central vein. On the right side of figure hepatocytes with vacuolar degeneration (green arrow) and pyknotic nuclei (black arrow) were observed, (f); section from rats treated with HDF + DEX + MET + FLU showed many hepatocytes with vacuolar degeneration (black arrow). mild dilatation and congestion of blood sinusoids (arrow head) is also noticed.

shunting [33,34]. These essentials were confirmed by changani et al., who stated that increase of blood glucose is due to gluconeogenesis [34].

In the current study the histological examination of liver tissues of HDF + DEX + FLU (20, 40) groups showed a massive fibrosis and degeneration of vacuolar of hepatocytes. These Findings agreed with what reported in Rodriguez and Acosta, and Khoza et al., about hepatotoxicity resulted from administration of fluconazole [35,36].

Serum levels of ALT and AST were elevated in a very pronounced way in HDF+DEX+FLU (20,40) groups in comparison with all other groups. Their levels were also increased significantly in HDF+DEX treated rats but still less than those results of groups that administered concomitantly with fluconazole. These results were in agreement with the work of Jackson et al., [31]. Studies showed that elevated levels of ALT and AST are considered as strong markers of insulin resistance where hyperinsulinemia leads to increase ALT and AST [37,38].

In the present study levels of serum Cytochrome P450 1A2 (CYP1A2) were elevated significantly in rats treated with HDF+DEX. Groups that treated with HDF + DEX + MET and HDF + DEX + FLU (20, 40) showed significant decrease in the concentration of serum CYP1A2 when compared with HDF + DEX treated rats. This decrease in the level of CYP1A2 may be attributed to the inhibitory effect of fluconazole on mammalian's CYP450 despite that fluconazol is more selective inhibitor of fungal CYP450 in comparison with other azoles [15,39]. These results could also be due to the increase of the levels of serum insulin in HDF+DEX+FLU (20, 40) groups, as insulin has role in down regulating CYP450 expression [40]. In the present study groups which received metformin with fluconazole HDF + DEX + MET + FLU (20, 40) showed serum level of CYP1A2 returned approximately to the normal level after its pronounced elevation caused by dexamethasone alone in HDF+DEX group. These findings were in line with other studies reported that metformin has a role in down regulation of certain isoforms of CYP450 expression [41-43].

Conclusion

Since fluconazole is considered as the first-line treatment in many fungal infections and the incidence of these infections is positively correlated with insulin resistance and with the impact of dexamethasone on the immune system, hence the combined administration of fluconazole and dexamethasone is very likely to happen in clinical practice. Therefore the current study revealed that fluconazole in both doses (20 and 40) mg/kg augmented the hepatotoxicity and insulin resistance effects that caused by dexamethasone. Thus, it is concluded that dose adjustment and monitoring are required in case of concomitant administration of fluconazole with dexamethasone for long duration of time.

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الفلوكونازول، مثبط إنزيم السيتوكروم P٤٥٠ للفطريات: الدور المحتمل في زيادة سمية الكبد ومقاومة الأنسولين المحدثة بالديكساميثازون في الجرذان

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مقاومة الأنسولين تعتبر مشكلة صحية واسعة الانتشارتظهر فى عدم أستجابة الخلايا للأنسولين وذلك يؤدي بدوره إلى زيادة معدل السكر بالدم وأيضا يزيد نسبة حدوث الالتهابات الفطرية. أستخدام الديكساميثازون لفترات طويلة يعتبر دواء هام لكثير من الحالات المرضية ولكنه بدوره يؤدى لحدوث مقاومة للأنسولين و حدوث للألتهابات الفطرية.

يعتبر الفلوكونازول من أوائل العقاقير المستخدمة لعلاج الفطريات ويعمل عن طريق تثبيط إنزيم السيتوكروم P٤٥٠ ولكن له تأثير سلبي على وظائف الكبد كما أنه يثبط بشكل جزئي إنزيمات السيتوكروم P٤٥٠ البشري أيضا وليس الفطري فقط. وهذا يؤثر على سرعة امتصاص وتأثير الأدوية، ومن هذه الأدوية الميتفورمين المستخدم لعلاج مقاومة الأنسولين.

تهدف هذه الدراسة إلى تقييم التأثيرات الناجمة عن تناول الفلوكونازول على وظائف الكبد ومستوى السيتوكروم P٤٥٠ ومقاومة الأنسولين المحدثة تجريبيا فى الجرزان بإعطاء الديكساميثازون مع إعطاء نظام غذائى عالي الدهون.

تم اعطاء الجرزان غذاءعالي الدهون لمدة ثمانية أسابيع ثم تم إحداث مقاومة الأنسولين للجرذان عن طريق حقن ديكساميثازون تحت الجلد (٠,١ مجم / كجم) لمدة أسبوعين و استمر أعطاء الغذاءعالي الدهون للجرزان حتى نهاية التجربة (عشر أسابيع).

تم أعطاء الفلوكونازول عن طريق الفم (٢٠ ، ٤٠ مجم / كجم) بالتزامن مع الديكساميثازون لمدة أسبوعين. كما تم إعطاء الميتفورمين (علاج مقاومة الأنسولين) عن طريق الفم أيضا لمدة أسبوعين بالتزامن مع ديكساميثازون بالأضافة للفلوكونازول بجرعة (٤٠٠ مجم/كجم).

مجموعات الجرزان التي تم أعطاؤها الفلوكونازول بجرعتيه مع الديكساميثازون أظهرت زيادة واضحة في مقاومة الإنسولين و إنزيمات اللانين أمينو ترانسفيراز والأسبرات أمينو ترنسفيراز مقارنة بمجموعة الجرذان الطبيعة. كما أظهرت نفس المجموعات انخفاضًا ظاهرا في مستوى إنزيم سيتوكروم P٤٥٠ وذلك مقارنا بالمجموعات التي حقنت الديكساميثازون منفردا والذى أدى بدوره لارتفاع تركيز إنزيم سيتوكروم P٤٥٠ ارتفاعا واضحا بالمقارنة مع الجرذان الطبيعية.

كما أظهرت الدراسة الهستيوباثولوجية حدوث تليف في أنسجة الكبد في الجرذان التي تناولت الفلوكونازول بجرعتيه تزامنا مع ديكساميثازون.

نتائج هذا البحث اثبت أن تناول عقار الفلوكونازل للجرزان المحدث لها مقاومة الأنسولين بإستخدام ديكساميثازون مع الغذاء عالى الدهون أدى إلى حدوث تفاعلات حيوية كيميائية نتج عنها زيادة في مستوى مقاومة الأنسولين ومستوى إنزيمات الكبد واضطراب في مستوى السيتوكروم P٤٥٠، مما أدى إلى تقليل الفعالية العلاجية للمتفورمين.