



## The Effects of Itraconazole on the Lipid Profile in Humans and Rabbits

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## ABSTRACT

**Objectives:** To investigate the effects of the azole antifungal agent, itraconazole, on the lipid profile of patients attending the dermatology clinics and any changes in serum creatinine associated. Also we aim at investigation of lipid profile of normo-lipidemic rabbits and any changes in serum creatinine associated with the use of these antifungal agents in the experimental animals and compare the outcome between humans and rabbits. **Methods:** In this study the effects of the antifungal drugs, itraconazole on the levels of serum lipids (triglycerides, cholesterol, high density lipoproteins and low density lipoproteins) and serum creatinine were investigated in humans and rabbits. Blood samples were taken before and 1 week following drug treatment. Blood samples were analyzed using commercially available kits. Treatment with itraconazole (200 mg/day) for one week in humans caused significant reductions in serum triglycerides, total cholesterol and LDL-cholesterol levels without any statistically significant alterations in HDL-cholesterol levels. In animal experiments blood samples were taken before and one, four, and six weeks following treatment with drugs. Blood samples were analyzed using commercially available kits. **Results:** Treatment with itraconazole 1 week to humans produced no significant changes in serum creatinine. The present results indicate that itraconazole, when used in therapeutic doses in humans, produced a significant effect on the levels of serum lipids except that of HDL-cholesterol. Treatment of rabbits with itraconazole (40 and 80 mg/kg/day) for six weeks produced a significant reduction in serum triglycerides and total cholesterol levels. However, its effects on HDL-cholesterol and LDL-cholesterol were not statistically significant. Treatment with itraconazole for six weeks in rabbits produced no significant changes in serum creatinine. **Conclusion:** The present results show clearly that itraconazole may have beneficial effects in patients who are suffering from hyperlipidemia and are in need of itraconazole for treatment of fungal infestations. Furthermore, when itraconazole is simultaneously prescribed together with lipid lowering drugs especially HMG-CoA reductase inhibitors such as lovastatin the level of creatine kinase, aldolase, lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase must be continuously monitored to avoid rhabdomyolysis. However, itraconazole, when used in therapeutic doses, produced significant reductions in the serum lipids of rabbits without any significant changes on the HDL or LDL-cholesterol levels.

**Keywords:** Itraconazole; Lipid profile; Humans; Rabbits

## 1. INTRODUCTION

Itraconazole is an orally administered triazole antifungal agent (Khoza, 2017). It exhibits excellent activity against most human fungal pathogens (Deuschle, 2004). The effects of ketoconazole as an inhibitor of cholesterol synthesis were studied on serum cholesterol and lipoproteins in five patients with prostate cancer who were treated with large doses of ketoconazole (1.2g/day orally), (Purohit, 2013). Serum total cholesterol and triglycerides, and very low density lipoprotein (VLDL), LDL, and HDL cholesterol were determined (Comte-Perret, 2014).

In rats, repeated dosing with itraconazole for three months produced a dose-related increase in serum cholesterol values. This effect was apparently not only dose-dependent, but also rat-specific, as the effect was not observed in either mice or dogs (Cetinkaya, 2005). In addition, no adverse effect on serum cholesterol levels was seen in patients, with either normal serum cholesterol values or pre-existing hypercholesterolaemia, who were treated with itraconazole at doses of up to 400mg/day. This indicates that the finding in rats is clinically irrelevant (Velegraki, 1996).

From the present review it appears that ketoconazole has a hypocholesterolemic potential. Itraconazole, a triazole derivative caused hypertriglyceridemia as a side effect (Bradbury, 2002). However, there is a lack of information regarding the effect of this drug on other lipid parameters in rabbits. Because of that it was felt necessary to investigate in detail the effect of this antifungal drug namely the azole itraconazole on the lipid profile in patients attending KKHU dermatology clinics as well as in rabbits.

## 2. MATERIAL AND METHODS

### Patients

From the dermatology clinic, of a tertiary university medical center, 1200 beds, during the period between January 2019 till March 2020, 55 patients with fungal infestation which required the use of itraconazole or terbinafine, were chosen. Forty patients were given itraconazole capsules orally. Terbinafine tablets were prescribed to the remaining 15 patients to be taken orally. The indication for which drugs be used, dose and duration of the antifungal treatment were determined by the treating physician. Patients on drugs that may increase or decrease serum lipids were excluded from the study. A fasting blood sample was obtained from the 55

patients before drug administration, and at one or two weeks post-treatment. The blood samples were analyzed for triglycerides, total cholesterol, HDL-C, LDL-C. so analysis of Biochemical Parameters were as follows:

#### **LDL-cholesterol determination**

The low density lipoprotein-cholesterol was quantitated by using the Friedwald (1972) formula as:

$$\text{LDL cholesterol} = \text{total cholesterol} - (\text{HDL cholesterol} + \text{triglyceride})/2.2$$

This is a simple and reliable method for estimating LDL-cholesterol and involves only the estimation of triglyceride, HDL-cholesterol and total cholesterol. It is widely used in clinical chemistry for LDL-cholesterol estimation.

#### **Triglyceride determination**

Lipids have presented an analytical problem in the past as they are insoluble and have large masses. The old methods for determining triglycerides involved extraction with organic solvents and tedious methodologies. However, with the advent of enzymatic methods direct estimation of serum or plasma lipids is carried out accurately.

#### **Total cholesterol determination**

The plasma level of cholesterol was measured by using BioMerieux enzymatic kits. This method estimates cholesterol as free cholesterol in the plasma according to the method previously described in a recent Study (Srisawasdi, 2012). Since in plasma almost 70% of the cholesterol is present in the form of esterified cholesterol, cholesterol esters were first converted to free cholesterol by cholesterol esterase.

#### **HDL-cholesterol determination**

HDL cholesterol was determined according to the method described in a recent Study (Engelking, 2015). The high density lipoproteins were separated and cholesterol bound to these fractions was estimated using BioMerieux HDL-cholesterol kit.

#### **Serum Creatinine Determination**

Creatinine will be determined according to the methods described in a recent Study. (Marakala, 2012) The plasma level of creatinine was measured by the BioMerieux enzymatic kit. (Sabbagh, 1988) Briefly, in the presence of strong bases such as NaOH, picrate reacts with creatinine to form a red chromophore. The rate of increasing absorbance at 492nm due to the formation of this chromophore is directly proportional to the creatinine concentration in the sample and is measured at 492nm.

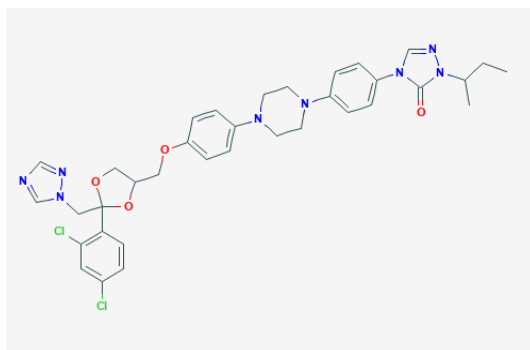
### **Experimental animals**

#### **Animals**

Sixty male, white rabbits of New Zealand strain, weighing 2.5 – 3 kg were obtained from the Animal Care Center of the College of Medicine, King Saud University, Riyadh.

#### **Drugs**

Itraconazole (Sporanox) was purchased as commercially available tablets from the market. Starch, lactose, and carboxymethyl cellulose were obtained from Sigma Chemical Company, St. Louis, M, USA (Figure 1).



**Figure 1.** Chemical structure of itraconazole

### **Administration of Drugs**

Itraconazole capsules were triturated (crushed and suspended) to 16 mg/ml/ and 32 mg/ml in 90 ml normal saline (0.9 NaCl) with 10 ml of carboxymethyl cellulose (CMC 1%) as a suspending agent.

Vehicle (starch 25 mg, lactose 115 mg, and magnesium hydrogen orthophosphate 5 mg suspended in 90 ml normal saline (0.9% NaCl) with 10 ml of CMC 1%.

### **Experimental Procedures**

The rabbits were housed in individual cages with free access to food and water in an air-conditioned room (20°C). The rabbits were divided into three groups of (10-13) animals each. Group I received itraconazole 40 mg/Kg/day, group II received itraconazole 80 mg/Kg/day, and group III received vehicle (lactose and starch suspended in carboxymethyl cellulose and normal saline).

Each drug was given to the corresponding group once daily via the oral route for six weeks (5 days/week). A fasting blood sample was obtained before drug administration, and at the end of the first, fourth, and sixth week. The central ear artery was cannulated with a polyethylene cannula 22 gauge for blood sampling. Blood was collected in venoject tubes. Serum samples were taken after centrifugation at 3500 rpm for 20 min for evaluating triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C). Analysis of Biochemical Parameters was done as follows:

- **LDL-cholesterol determination**

The low density lipoproteins were separated and cholesterol bound to these fractions was estimated using the BioMerieux LDL-cholesterol kit. The various classes of lipoproteins are differentiated according to their density, electrical behaviour and reactivity with specific antibodies. The addition of certain amphipathic polymers precipitated certain lipoprotein fractions specifically. There is a good correlation between the levels of cholesterol and phospholipids measured in the precipitated fractions and in the LDL isolated by ultracentrifugation.

- **Triglyceride determination**

Lipids have presented an analytical problem in the past as they are insoluble and have large masses. The old methods for determining triglycerides involved extraction with organic solvents and tedious methodologies. However, with the advent of enzymatic methods direct estimation of serum or plasma lipids is carried out accurately.

- **Total cholesterol determination**

The plasma level of cholesterol was measured by using BioMerieux enzymatic kits. This method estimates cholesterol as free cholesterol in the plasma according to the method previously described by an interventional study (Lolekha, 1996). Since in plasma almost 70% of the cholesterol is present in the form of esterified cholesterol, cholesterol esters were first converted to free cholesterol by cholesterol esterase.

- **HDL-cholesterol determination**

HDL cholesterol was determined according to the method described in an experimental study (Sivgin, 2014). The high density lipoproteins were separated and cholesterol bound to these fractions was estimated using BioMerieux HDL-cholesterol kit.

- **Serum Creatinine Determination**

Creatinine will be determined according to the methods described by many studies (Burke, 2019). The plasma level of creatinine was measured by the BioMerieux enzymatic kit. Briefly, in the presence of strong bases such as NaOH, picrate reacts with creatinine to form a red chromophore (Owen, 2007). The rate of increasing absorbance at 492nm due to the formation of this chromophore is directly proportional to the creatinine concentration in the sample and is measured at 492nm.

### **Statistical analysis**

The results of patient study are expressed as the mean+S.E.M and are presented in the form of tables. Statistical analysis of patient data were performed using the Student two-tailed t-test for matched pairs. P values < 0.05 were considered significant. The results of animal study are expressed as the mean±S.E.M and are presented in the form of bar chart and also tables. Statistical analysis of animal data was performed using the repeated measure ANOVA for comparison within the group; ordinary ANOVA for comparison between groups at the same duration of treatment. P values < 0.05 were taken as significant. Post ANOVA Tukey-Kraemer test was used them in assessment of significance between and within groups.

### 3. RESULTS

#### Patients

A total of 40 patients were enrolled for the study. 13 patients did not turn up after the first visit. The 17 patients who completed the study were 7 males and 10 females. Their age range was 16-58 years with an average of 37years.

#### **Effect of itraconazole on the concentrations of serum triglycerides, total cholesterol, HDL-cholesterol and LDL- cholesterol**

Treatment with itraconazole (200 mg/day) reduced the serum triglycerides, total cholesterol and LDL levels by 20.61, 8.46, and 13.17% respectively. HDL levels were not significantly changed (Table 1-4).

**Table 1:** Effect of itraconazole on the concentrations of serum triglycerides( mmol/l)

Variables	n	TG Pre-treatment (mmol/l)	TG Post-treatment (mmo/l)	P-value	(%) Change
TG	17	1.28±0.15	1.01±0.14 <sup>a</sup>	0.03	-20.61±7.49 <sup>a</sup>

Results represent the mean±SEM of 17 samples.

<sup>a</sup>p<0.05 (comparison using the paired t-test).

TG:triglycerides.

n: number of patients.

**Table 2:** Effect of itraconazole on the concentrations of total serum cholesterol(mmol/l)

Variables	n	TC Pre-treatment (mmol/l)	TC Post-treatment (mmol/l)	P-value	(%) Change
TC	17	5.32±0.31	4.81±0.26 <sup>a</sup>	0.009	-8.46±2.84 <sup>a</sup>

Results represent the mean±SEM of 17 samples.

<sup>a</sup>p<0.05 (comparison using the paired t-test).

TC: total cholesterol.

n: number of patients.

**Table 3:** Effect of itraconazole on the concentrations of serum HDL-cholesterol(mmol/l)

Variables	n	HDL Pre-treatment (mmol/l)	HDL Post-treatment (mmol/l)	P-value	(%) Change
HDL	10	1.29±0.09	1.19±0.06	0.32	-4.14±7.29

Results represent the mean±SEM of 10 samples.

<sup>a</sup>p<0.05 (comparison using the paired t-test).

HDL: high density lipoproteins.

n: number of patients

**Table 4:** Effect of itraconazole on the concentrations of serum LDL-cholesterol(mmol/l)

Variables	n	LDL Pre-treatment (mmol/l)	LDL Post-treatment (mmol/l)	P value	(%) Change
LDL	10	3.96±0.37	3.36±0.28 <sup>a</sup>	0.024	-13.17±4.43 <sup>a</sup>

Results represent the mean±SEM of 10 samples.

<sup>a</sup>p<0.05 (comparison using the paired t-test).

LDL: low density lipoproteins.

n: number of patients.

### Effect of itraconazole on the concentrations of serum creatinine

Itraconazole produced no significant effects on the concentrations of serum creatinine in humans (Table 5).

**Table 5:** Effect of itraconazole on the concentration of serum creatinine( $\mu\text{mol/l}$ )

Variables		Creatinine Pre-treatment ( $\mu\text{mol/l}$ )	Creatinine Post-treatment ( $\mu\text{mol/l}$ )	P-value	% change
creatinine	8	83.12 $\pm$ 7.27	88.06 $\pm$ 4.25	0.36	9.46 $\pm$ 7.03

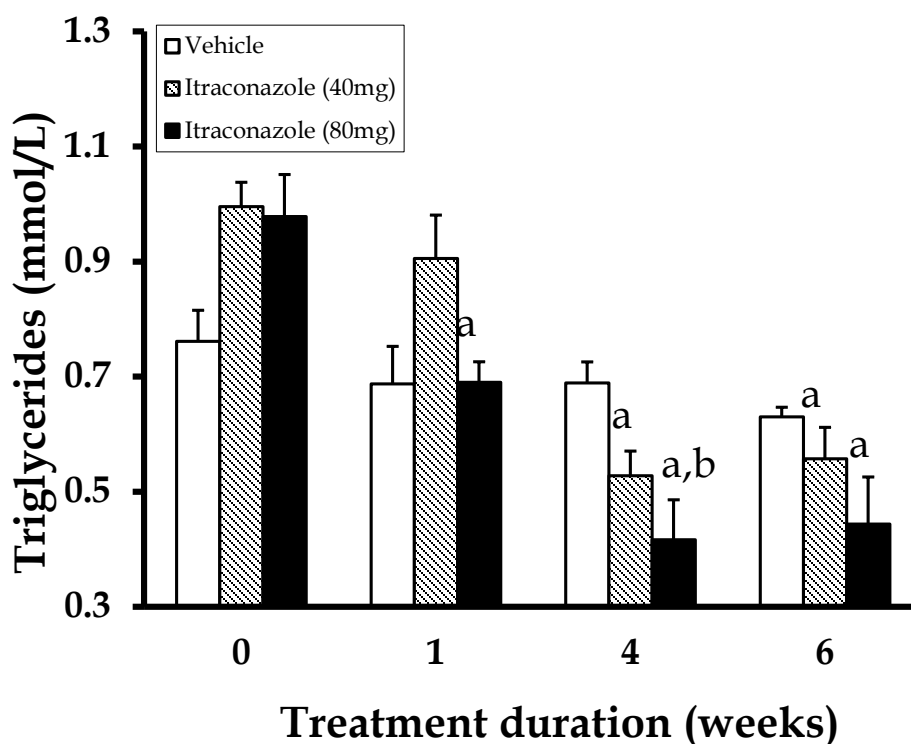
Results represent the mean $\pm$ SEM of 8 samples.

n: number of patients.

### Animals

#### Effect of itraconazole on the concentrations of serum triglycerides

Itraconazole (40 mg/kg/day) when it was given orally for six weeks produced a reduction in serum triglycerides concentration which started from the first week following treatment. A highly significant reduction in the triglycerides level ( $p < 0.001$ ) was observed in the group of rabbits which had been treated with itraconazole (40 mg/kg/d) orally for six weeks as compared to the pretreatment level but not to vehicle (starch + lactose + magnesium sulphate in 1% carboxy methyl cellulose CMC) treated group following four and six weeks of treatment (Figure 2).



**Figure 2:** The effect of itraconazole on the concentrations of serum triglycerides in rabbits

Data represent the mean $\pm$ SEM. Vehicle (starch+lactose+magnesium sulphate) and itraconazole were administered daily by the oral route for six weeks.

<sup>a</sup> $p < 0.05$  as compared to pretreatment levels (comparison within same group by using repeated measure ANOVA).

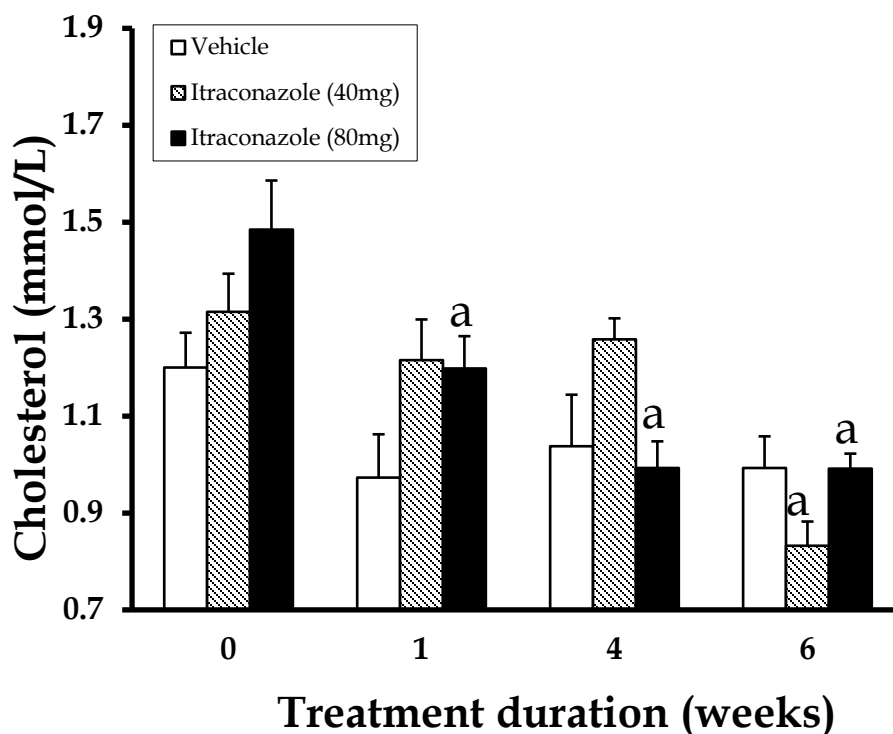
<sup>b</sup> $p < 0.05$  as compared to vehicle ( comparison between groups at the same duration of treatment by using ordinary ANOVA).

Similarly, itraconazole (80 mg/kg/day) when it was administered orally for six weeks produced significant reductions in serum triglycerides concentrations ( $p < 0.01$ ) but the effect was more pronounced after the first week of treatment, ( $p < 0.001$ ) at the fourth and sixth week of treatment as compared to pretreatment level. The changes in serum triglycerides level in the first and sixth week produced by itraconazole (80 mg/kg/day) were statistically significant in comparison to the pretreatment levels of the same animals

but not to the vehicle-treated group. Also, these changes were statistically significant in comparison with the vehicle ( $p < 0.01$ ) and the pretreatment level ( $p < 0.001$ ) following four weeks of treatment.

### **Effect of itraconazole on the concentrations of total serum cholesterol**

Itraconazole (40 mg/kg/day) orally when administered for six weeks produced a reduction in total serum cholesterol concentration which was not statistically significant as compared with the vehicle. The reductions produced by itraconazole were only significantly different following the sixth week of treatment ( $p < 0.001$ ) as compared to pretreatments level in the same animals. On the other hand, mean total serum cholesterol levels fell after one week of treatment with itraconazole (80 mg/kg/d orally) for six weeks which was statistically significant compared to pretreatment level ( $p < 0.05$ ) but not significant as compared to vehicle. Itraconazole (80 mg/kg/d) produced further reduction in total serum cholesterol levels following four and six weeks of treatment. This reduction was statistically significant as compared to pretreatment levels ( $p < 0.001$ ) but not significant as compared to vehicle (Figure 3).



**Figure 3:** The effect of itraconazole on the concentrations of total serum cholesterol in rabbits

Data represent the mean  $\pm$  SEM. Vehicle (starch+lactose+magnesium sulphate) and itraconazole were administered daily by the oral route for six weeks.

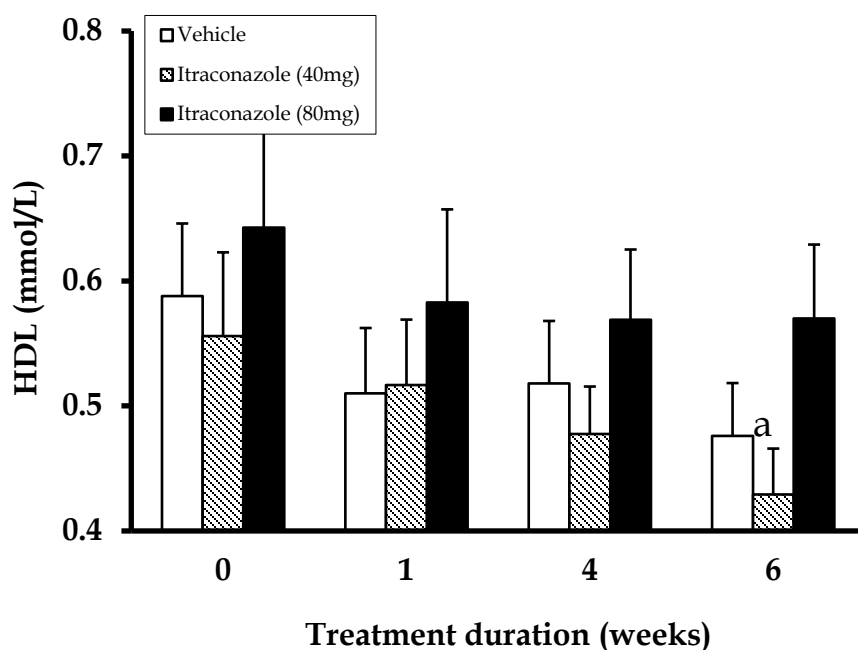
<sup>a</sup> $p < 0.05$ , as compared to pretreatment levels (comparison within same group by using repeated measure ANOVA).

### **Effect of itraconazole on the concentrations of serum HDL-cholesterol**

Itraconazole (40 mg/kg/d) produced a reduction in serum HDL-cholesterol concentrations, which was not statistically significant following one or four weeks of treatment. However it was statistically significant following six weeks of treatment as compared to pretreatment levels ( $p < 0.05$ ) but not significant as compared to vehicle (Figure 4). Itraconazole (80 mg/kg/d) produced no significant effect on serum HDL-cholesterol concentrations.

### **Effect of itraconazole on the concentrations of serum LDL-cholesterol**

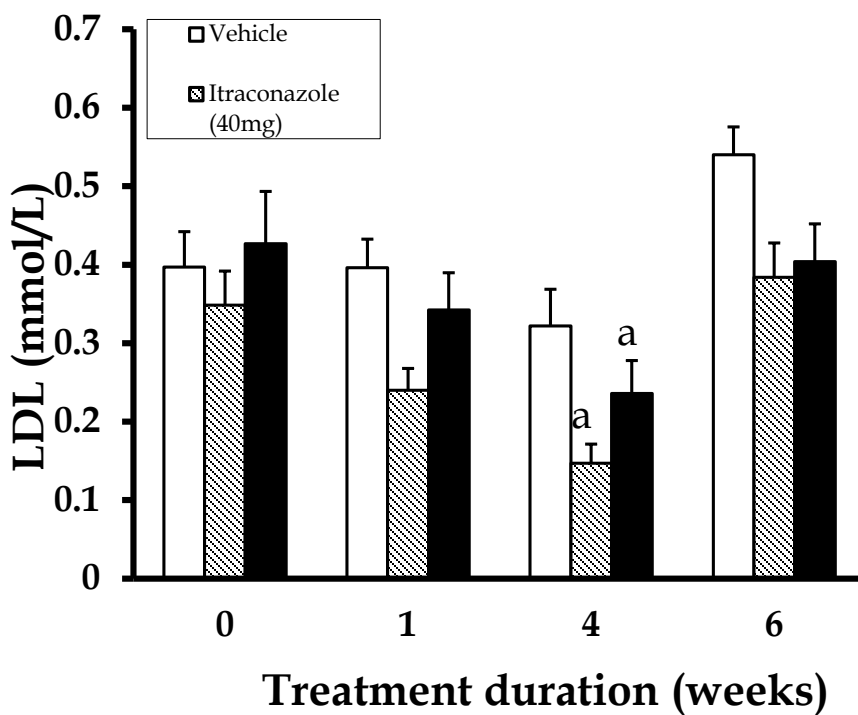
Itraconazole (40 or 80 mg/kg/day) did not produce any significant changes in LDL-cholesterol levels as compared to vehicle. Itraconazole (40 mg/kg/day) caused a significant reduction in serum LDL-cholesterol levels following four weeks of treatment as compared to pretreatment levels ( $p < 0.01$ ) but this effect did not persist. Following six weeks of treatment, however, LDL-cholesterol levels returned to near pretreatment values (Figure 5).



**Figure 4:** The effect of itraconazole on the concentrations of serum HDL-cholesterol in rabbits

Data represent the mean  $\pm$  SEM. Vehicle (starch+lactose+magnesium sulphate) and itraconazole were administered daily by the oral route for six weeks.

<sup>a</sup>p < 0.05, as compared to pretreatment level (comparison within same group by using repeated measure ANOVA).



**Figure 5:** The effect of itraconazole on the concentrations of serum LDL-cholesterol in rabbits

Data represent the mean  $\pm$  SEM. Vehicle (starch+lactose+magnesium sulphate) and itraconazole were administered daily by the oral route for six weeks.

<sup>a</sup>p < 0.05, as compared to pretreatment level (comparison within the same group by using repeated measure ANOVA).



Similarly, itraconazole (80 mg/kg/day) produced significant reduction in the serum LDL-cholesterol levels ( $p < 0.01$ ) as compared to pretreatment level. This effect was observed only following four weeks of treatment (Figure. 5).

Effects of itraconazole on serum lipids are summarized in table 6. In table 7 the same data is presented but are expressed as a mean percent change. Itraconazole (40 mg/kg/day orally) when it was administered for six weeks produced significant reduction in the serum triglycerides concentrations as compared with vehicle. Mean percent reduction in triglycerides after four and six weeks of treatment were 46.3% (compared to mean percent change at the first week i.e within group,  $p < 0.001$ ; compared to vehicle,  $p < 0.001$ ) and 43.9% (within group,  $p < 0.001$ ; compared to vehicle,  $p < 0.05$ ) respectively.

**Table 6:** The effect of itraconazole on the concentrations of serum lipid (mmol/l) in rabbits

Parameter	Duration of treatment (weeks)											
	0			1			4			6		
	Itraconazole (mg/kg/day, p.o.)											
	Veh	40	80	Veh	40	80	Veh	40	80	Veh	40	80
TG	0.76 ±0.02	0.99 ±0.04	0.98 ±0.07	0.69 ±0.07	0.91 ±0.08	0.69 ±0.04 <sup>a</sup>	0.69 ±0.04	0.53 ±0.04 <sup>a</sup>	0.42 ±0.07 <sup>a,b</sup>	0.63 ±0.02	0.56 ±0.05 <sup>a</sup>	0.44 ±0.08 <sup>a</sup>
TC	1.20 ±0.07	1.32 ±0.08	1.50 ±0.10	0.97 ±0.09	1.22 ±0.08	1.20 ±0.07 <sup>a</sup>	1.04 ±0.11	1.26 ±0.04	0.99 ±0.06 <sup>a</sup>	0.99 ±0.07	0.83 ±0.05 <sup>a</sup>	0.99 ±0.03 <sup>a</sup>
HDL	0.59 ±0.06	0.56 ±0.07	0.64 ±0.08	0.51 ±0.05	0.52 ±0.05	0.58 ±0.07	0.52 ±0.05	0.48 ±0.04	0.57 ±0.06	0.48 ±0.04	0.43 ±0.04 <sup>a</sup>	0.57 ±0.06
LDL	0.39 ±0.04	0.35 ±0.04	0.43 ±0.07	0.39 ±0.04	0.24 ±0.03	0.34 ±0.05	0.32 ±0.05	0.15 ±0.02 <sup>a</sup>	0.24 ±0.04 <sup>a</sup>	0.54 ±0.04	0.38 ±0.04	0.40 ±0.05

Data represent the mean ± SEM of 9-13 rabbits. Itraconazole (40 or 80 mg/kg orally) and vehicle (starch+lactose+magnesium sulphate) were administered once daily for 6 weeks.

<sup>a</sup> $p < 0.05$ , as compared to pretreatment levels (comparison within the same group by using repeated measure ANOVA).

<sup>b</sup> $p < 0.05$ , as compared to vehicle (comparison between groups at the same duration of treatment by using ordinary ANOVA).

Legend: TG = triglycerides; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein;

Veh=vehicle

**Table 7:** The effect of itraconazole on the concentrations of serum lipid (mmol/l) in rabbits (expressed as the mean percent change)

Parameter	Duration of treatment (weeks)								
	1			4			6		
	Itraconazole (mg/kg/day, p.o.)								
	Veh	40	80	Veh	40	80	Veh	40	80
TG	-9.92 ±8.28	-6.99 ±8.72	-25.49 ±6.81	-8.93 ±5.38	-46.30 ±4.91 <sup>a,b</sup>	-56.32 ±7.29 <sup>a,b</sup>	-17.20 ±1.67	-43.90 ±5.36 <sup>a,b</sup>	-54.78 ±7.95 <sup>a,b</sup>
TC	-19.01 ±5.68	-6.77 ±5.03	-16.98 ±4.69	-13.70 ±7.19	-1.91 ±4.38	-31.04 ±4.69 <sup>a</sup>	-13.10 ±7.31	-33.50 ±6.54 <sup>a</sup>	-29.14 ±5.84
HDL	-0.936 ±10.9	-0.37 ±8.01	-9.34 ±4.65	-9.14 ±8.45	-5.73 ±7.37	-6.79 ±6.27	-16.30 ±6.76	-16.00 ±7.37	-6.44 ±7.19
LDL	5.53 ±8.25	-15.5 ±13.7	-8.51 ±10.9	-9.03 ±15.9	-42.50 ±13.2	-38.16 ±8.79	58.61 ±25.9	33.24 ±20.1 <sup>a</sup>	19.33 ±25.8

Data represent the mean percent change ± SEM of 9-13 rabbits. Itraconazole (40 or 80 mg/kg orally) and vehicle (starch+lactose+magnesium sulphate) were administered once daily for 6 weeks.

<sup>a</sup> $p < 0.05$ , as compared to 1 week of treatment (comparison within the same group by using repeated measure ANOVA).

<sup>b</sup> $p < 0.05$ , as compared to vehicle (comparison between groups at the same duration of treatment by using ordinary ANOVA).

Legend: TG = triglycerides; TC = total cholesterol; HDL = high density lipoprotein; LDL = low density lipoprotein;

Veh=vehicle

Treatment with itraconazole (40 mg/kg/day) did not produce any significant changes in the total serum cholesterol or LDL levels as compared to the respective values following treatment with the vehicle. Itraconazole (40 mg/kg/day) reduced the mean serum levels of total cholesterol following 6 weeks of treatment by 33.5%. The level of significance within the group,  $p < 0.001$ ; while that of drug treated as compared to vehicle was not significant. It also increased LDL levels by 33.24% ( $p < 0.01$ , comparison was made within the group and it was not significant when the drug treated animals were compared to vehicle).

These significant changes were observed following the sixth week of treatment as compared to mean percent change in the first week. Itraconazole (40 mg/kg/day) did not affect the HDL-cholesterol levels (Table 7).

Itraconazole (80 mg/kg/day) produced a significant reduction in the mean serum levels of triglycerides by 56.32% following four weeks of treatment. The level of significance, within the group and as compared to the vehicle, was  $p < 0.001$ . It also produced significant reduction in mean serum levels of triglycerides by 54.78% following six weeks of treatment. The level of significance, within the group and as compared to the vehicle, was  $p < 0.001$ . It also reduced the mean serum level of total cholesterol by 31.04% following four weeks of treatment. The level of significance, within the group was  $p < 0.05$  and as compared to the vehicle was not significant. Mean LDL-cholesterol serum level was also reduced by 38.16% in the fourth week of treatment but this was not statistically significant. It had no significant effects on the serum levels of HDL-cholesterol.

### **Effect of itraconazole on the concentrations of serum creatinine**

Itraconazole (40 and 80 mg/kg/d) produced no significant effects on the concentrations of serum creatinine in rabbits (Table 8).

**Table 8:** The effect of itraconazole on the concentration of serum creatinine ( $\mu\text{mol/l}$ ) in rabbits

Treatment	Dose (mg/kg/day p.o.)	n	Duration of treatment			
			0	1 wk	4 wks	6 wks
Vehicle		11	128.64 $\pm 10.15$	112.09 $\pm 11.09$	129.45 $\pm 5.24$	131.36 $\pm 2.65$
Itraconazole	40	11	133.59 $\pm 3.09$	131.95 $\pm 6.94$	139.64 $\pm 1.67$	130.78 $\pm 4.09$
Itraconazole	80	11	116.55 $\pm 9.53$	113.05 $\pm 8.81$	125.36 $\pm 6.86$	131.18 $\pm 3.84$

Data represent the mean  $\pm$  SEM. Vehicle (starch+lactose+MgHPO<sub>4</sub>) and itraconazole were administered daily the via oral route for six weeks.

n: number of animals used.

wks: weeks

## 4. DISCUSSION

Researchers and clinicians have recognized hypertriglyceridaemia as a possible risk factor for development of IHD for over 30 years (Carlson, 2009; Blake, 2004; Criqui, 2009; Toth, 2012). A large meta-analysis of population-based, prospective studies demonstrated that triglyceride levels are a predictor of CHD (Khare, 2016), even after adjusting for low HDL and other risk factors. In a recent Study (He, 2004), there was a gradient increase in rates of CHD as triglyceride levels increased, even after adjustment for major CHD risk factors, including high LDL cholesterol levels. Recent data from studies controlling for HDL have tended to support a clinically relevant interaction between cholesterol and triglycerides in assessing the risk of coronary heart disease.

In a cohort study, serum triglyceride concentrations were a strong and independent predictor of outcome (myocardial infarction) over seven years of follow up, independently of HDL (Nozue, 2016). Further evidence comes from a meta-analysis incorporating data from eight populations based prospective studies in over 28000 patients (about 80% male) and controlling for HDL. This analysis showed that for every 1 mmol/l increase in serum triglyceride concentration the relative risk of CHD increased by 14% in men and 37% in women (Burke, 2019). Therefore, hypertriglyceridaemia has a detelerious effect on the body.

The present study demonstrates that itraconazole produces a significant reduction in serum triglyceride concentrations in humans. It has been reported in a recent study (Cetinkaya, 2005). That itraconazole therapy produced hyper-triglyceridaemia in patients who were treated with this drug at a dose of 50-400 mg/day for a period of 5 months for the treatment of a variety of systemic mycosis. Although, it appears that the results of this study are dissimilar to our present data but this was not a properly

conducted study (Kea, 2018; Purohit, 2013). The hypertriglyceridaemia was only observed as a side effect in some patients who had taken the medication (Malamisura, 2009; Alireza, 2011; Carmina, 2003). In the present study, itraconazole produced significant reduction in serum total cholesterol concentrations in humans. Similar results have been reported in another study (Ranawaka, 2015).

In humans, itraconazole did not produce significant effects on serum HDL-cholesterol concentrations. There are no published reports on the effect of itraconazole on serum HDL-cholesterol concentrations in humans. The effects of other imidazole-derived drugs such as ketoconazole on serum HDL-cholesterol concentrations in humans had been reported. The imidazole antifungal drug, ketoconazole have been reported to have no effect on HDL-cholesterol serum concentrations (Meikle, 2011). The results of these studies are consistent with our present finding regarding the effect of the triazole, itraconazole, on the serum HDL-cholesterol concentrations. Other investigators have reported on the effects of itraconazole on serum total cholesterol concentrations in rats (Vidyasagar, 2016). The imidazole antifungal drugs, ketoconazole, have been reported to decrease serum LDL-cholesterol levels (Fleseriu, 2016; Cedeno, 2012). The results of these studies are consistent with our present findings regarding the effect of the triazole, itraconazole, on the serum LDL-cholesterol concentrations (Sabzghabae, 2009).

Furthermore, it had been shown by a recent study that treatment with terbinafine or itraconazole did not produce any clinically significant changes in the tests of liver and kidney function (Hay, 2006). This was a comparative study to evaluate the efficacy of these two drugs when they were given for the treatment of tinea pedis for a period of 2 weeks. Our study was further supported by other studies that compared the effects of terbinafine and itraconazole on routine laboratory tests (Bilgili, 2015; Succi, 2013). They found that both drugs had no significant effects on the routine laboratory tests including serum creatinine. It had also been demonstrated that treatment of humans with terbinafine had no significant effects on serum creatinine concentrations (Friedlander, 2002; Kumar, 2014). The study had some limitations which were considered as shortcomings and it is recommended to be overcome later on in future studies, such as small sample size. Work was done with poor sample size while correlating with previous works, and it may affect the accuracy of results. But as it was a clinical trial, so it was very difficult to attain a large sample size because of lack of resources and depending on the powerful study design it appeared to us that this sample size was perfect.

## 5. CONCLUSION

In humans, it caused significant reduction in serum triglycerides, total cholesterol, and LDL-cholesterol concentrations following 1 week of treatment. In animals itraconazole caused significant reduction in serum triglycerides and total cholesterol concentrations following 6 weeks of treatment. There were significant lowering effects of itraconazole on serum triglycerides and total cholesterol in rabbits.

### Abbreviations

LDL; low density lipoproteins, HDL; high density lipoproteins, CHD; coronary heart disease, VLDL; very low density lipoproteins.

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### Author Contributions

Abdulmajeed Alajlan, was the first and corresponding author who elicited and generated the idea, started and supervised the work. Sami Alsuwaidan, and Ali Mustafa, contributed to data collection and analysis. Omar Alshiekh, and Huda Alkreathy wrote the manuscript.

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This study has not received any external funding.

### Conflict of Interest

The authors declare that there are no conflicts of interests.

### Informed consent

Written & Oral informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this manuscript.

### Ethical approval for human

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (ethical approval number 98-723j/h9\_b\_v).

### Ethical approval for animal studies

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted (ethical approval number 789-hh9/03\_4t\_j/kn).

### Data and materials availability

All data associated with this study are present in the paper.

### Peer-review

External peer-review was done through double-blind method.

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