

# A COMPARATIVE STUDY OF ORAL AND TOPICAL KETOCONAZOLE IN THE TREATMENT OF PITYRIASIS VERSICOLOR



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### Keywords:

ketoconazole,  
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## ABSTRACT

**Objective:-**The first purpose of the present work is in vivo (clinical study): A comparative study of oral and topical Ketoconazole in the treatments of P V and (second) in vitro (laboratory study): To determine the activity of Ketoconazole against *M Furfur* by (MIC) test.

**Study patients:-** In vivo or clinical study included only one hundred and seventy eight outpatients. Each patient was examined clinically before treatment as well as by laboratory examination.. After treatment, cure was confirmed by wood's light examination , 95 % of cases were no fluorescence while direct microscopic examination showed cure of first degree 64 % , 44 % , 40 % , 12 % in groups A , B , C , D respectively for yeast and for hyphae cure was 100% , 100% , 100 % , 84 % in groups A , B , C , D respectively.

**Results: -** With regard antifungal susceptibility test (MIC), our results showed that Ketoconazole was highly effective against 40 isolates of the fungus with MIC ranging from 0.03 – 0.5 µg/ml. MIC50 was 0.06 µg/ml and MIC90 was 0.25 µg/ml. However drug acts as fungicidal action (MFC) in 19 (47.5 %) of isolates and fungistatic action (MIC) in 21 (52.5 %) of isolates. The study concluded that ketoconazole was highly effective in vivo and in vitro., clinical trial reported the great cure in group A (200mg) followed by group B (400mg), then group C (2% cream) and group D (2% shampoo) was the less cure. Also, great side effect in group A followed by C, D then B.

## INTRODUCTION:

Pityriasis versicolor (PV) is a chronic fungal infection of the stratum corneum. The dimorphic fungus of *Malassezia furfur* (*Pityrosporum orbiculare*) is the infecting organism. This organism is more commonly seen in areas of the skin with sebum production and infection is seen more commonly in adolescents and young adults. Pityriasis versicolor (PV) is present with a lesion of varying color depending on the individual's skin type. In lighter skinned individuals, the lesions are typically seen as reddish – brown macules with fine scales .

In darker skinned individuals, the lesions may appear as hyperpigmented or hypopigmented macules. The common locations of these lesions are the neck, upper chest, upper back and upper arm<sup>1,2</sup>.

Treatment of pityriasis versicolor (PV) involves topical and oral therapy; when topical therapy fails, oral therapy can be used. Ketoconazole, fluconazole and itraconazole are commonly used in oral therapy. The main problem with the use of topical antifungal is the difficulty to apply cream to such a wide body surface area<sup>3</sup>. Oral therapy is not without risk, the decision to treat with an oral agent should be made only after a complete discussion of the risk

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involved. Ketoconazole is an imidazole antifungal agent .It is formulated as tablet, cream and shampoo which are used in the treatment of pityriasis versicolor (PV)<sup>4</sup>. This study has been undertaken to compare clinically three different dosage forms (tablet, cream and shampoo) and two oral doses of ketoconazole clinically. Further, to perform haematological parameters before and after treatment to determine the effect of infection with the *Malassezia furfur* and the effect of treatment with the ketoconazole respectively. Furthermore, to isolate *Malassezia furfur* from patients and determining the MIC and MFC of ketoconazole in vitro to answer the question "is treatment failure due to development of resistance or inadequacy of treatment?"

## PATIENTS AND METHODS

### STUDY PATIENTS:-

A total of one hundred and seventy eight out patients with PV attended to the Dermatology Department of AL-Ramadi General Hospital from May 2004 to May 2005. Only one hundred patients were included in this clinical trial, their age ranged between 13-60 years. Seventy three (73%) were males and 27 (27%) patients were females. A full history was taken from each patient according to the questionnaire, about age, gender (males and females), duration of the disease, occupation, family history and previous drug treatment. Clinical examination was performed on each patient in this study with the help of experient dermatologist, including general physical and skin examination.

Wood's light Examination was examined by wood's lamp in a dark room to see fluorescence of *M. furfur* under wood's lamp. Yellow fluorescent (+ve) with varying degree indicated the presence of fungus with a different severity, no fluorescent (-ve) means absence of fungus.

One hundred patients were included in this clinical trial. The selected patients were randomly divided into the following treatment groups Group A included twenty five patients (Twenty males and five females). They were treated with oral ketoconazole tablet (Nizoral)<sup>R</sup> (Janssen pharmaceutica), (Beerse, Belgium), (Expiry date July 2007), (Batch No. 02GB726).200 mg (one tablet of 200 mg ) once daily for 2 weeks. Patients were advised to take this drug with food. And group B which included twenty five patients (twenty males- and five females). They were treated with oral ketoconazole tablet (Nizoral)<sup>R</sup> (Janssen pharmaceutica), (Beerse, Belgium) (Expiry, date July2007), (Batch No. 02GB726). Four hundred (two tablets of 200 mg) once / week for 2 weeks. Patients were advised to take this drug with food. Group C which also included twenty five patients (eighteen males and seven females). They were treated with topical 2% ketoconazole cream (ketoconazole)<sup>R</sup> (Al-Shahba Labs), (Aleppo, Syria),(Expiry date April 2007), (Batch No. 9001) twice daily for 2 weeks. Finally, group D which also included twenty five patients (fiveteen males and ten females). They were treated with topical 2% ketoconazole shampoo (ketonaz)<sup>R</sup> (Domina pharmaceuticals), (Damascus, Syria) (Expiry date November 2005), (Batch No.0236230). Patients were advised to use ketoconazole 2% shampoo by applying to the affected area for 15 minutes, then bathing the treated area to be repeated for 3 consecutive days.

### LABORATORY PROCEDURES:-

After clinical diagnosis of PV for each patient. The specimens (scales) were taken from the infected area by scraping with a scalpel or by sterile glass slide. A few drops of 10% potassium hydroxide solution (KOH) were put on the glass slide and then scales were put with a gentle pressure and covered by cover

slip and heated gently for 5 minutes. Then slide was examined under light microscope<sup>5</sup>.

### **CULTURE OF FUNGUS:-**

Specimens were cultured on the sabouraud dextrose agar plates (as two sets). The surface of the agar inoculated with the specimen was covered with a thin layer of sterile olive oil to enhance the growth of fungus. These inoculated plates were incubated at 37C<sup>0</sup> for 2 weeks to isolate yeast form and at 25-30C<sup>0</sup> for 1-2 weeks to isolate hyphae form. Identification was carried out according to Kwon-chung and Bennett<sup>5</sup> and Edman<sup>6</sup>.

Biochemical investigations (liver function tests):

Liver function tests were done for each patients, they included the following tests:- total serum Bilirubin, serum pyruvate transaminase (SGPT) or alanine aminotransferase (ALT), alkaline phosphatase (ALP).

In Vitro Antifungal Susceptibility TEST (MINIMUM INHIBITORY

### **CONCENTRATION (MIC) TEST:**

Preparation of ketoconazole in different concentrations ranging from 16 µg/ml to 160 µg/ml was performed according to NCCLS<sup>7</sup>. The quality control (QC) in this test was *Candida albicans*. It was identified by growth rate, morphology of the organism and germ tube test<sup>8</sup>.

The same procedure was used to prepare the inoculum size of QC and *M. furfur* (test strain). It was achieved by picking 5 colonies (from pure culture ) at least 1mm in diameter and 5 ml sterile normal saline (Nacl 0.9%) added and mixed to obtain sterile suspension. Suspension was standarized to 0.5 Mcfarland scale (1-5x10<sup>6</sup> cfu/ml) by adding normal saline to suspension until the visually match occurred between suspension and Macfarland tube by using

spectrophotometer. One ml of suspension was add to 9 ml of RPMI -1640 broth media. Then, mixed to obtain (1-5 x 10<sup>5</sup> CFU / ml). It may be held at 2-8C<sup>0</sup> for up 3 hours. Final inoculum size (1-5x10<sup>4</sup>CFU/ml ) of QC and test strain was prepared by adding 1 ml of suspension (1-5x10<sup>5</sup> CFU/ml ) to 9 ml of RPMI – 1640 broth media (1: 10 dilution), and mixed well. The MIC for the antimicrobial agent against *Candida albicans* and *M. furfur* by using broth dilution method was achieved<sup>7,9</sup>.

MIC was determined as the least amount of antimicrobial that will inhibit visible growth of organisms after overnight incubation, while the minimum fungicidal concentration (MFC) is the lowest concentration of the agent that will prevent growth<sup>10</sup>.

### **RESULTS:**

All descriptive data of study were randomly distributed into four groups (A,B,C,D). The age of the patients treated ranged between 13-60 years. The males were 73 (73%) and females 27(27%) of the total number treated .Pityriasis versicolor was more common in males than females in a ratio of 2.7:1. A positive family history was obtained in 47(47%) of the cases while 53(53%) had a negative family history.

Our results showed that 67(67%) of cases were with greasy skin, 19(19%) with dry skin and 14(14%) with normal skin. (Table 4.2) shows results of skin type of four groups. In most patients, the most site affected on the body was back, chest, neck or shoulder. Patients with hyperpigmented macules were 43(43%), (Fig. 12,16) 40(40%) of hypopigmented lesion and 17(17%) of mixed type of lesion (hyper and hypopigmented macules) from the total cases. This study showed that duration of lesions ranged between (2 weeks to 25 years) for the total cases.

## WOOD'S LIGHT EXAMINATION

The study patients showed yellow fluorescence (+ve) of various degree before treatment in the group A,B,C,D. All these patients showed no fluorescence after treatment. While 5 (5%) of patients showed no fluorescence before and after treatment.

### Direct Microscopic Examination

#### A- Results of four groups (regimens)

##### Group A (oral ketoconazole, 200mg)

Cure regarding the yeast form was found to be 16(64%) of first degree, 7(78%) of second degree and 2(8%) of third degree. However all 25 patients showed negative scraping for the hyphae after treatment (cure of first degree for hyphae was 25(100%)

##### Group B (oral ketoconazole, 400 mg)

Cure regarding the yeast form was found to be 11(44%) of first degree, 12(48%) of second degree and 2(8%) of third degree. All 25 patients showed negative scraping for the hyphae form after treatment (cure of first degree of hypae was 25(100%).

##### Group C (topical ketoconazole, 2% cream).

Cure of the yeast form was found to be 10(40%) of first degree, 11(44%) of second degree and 4(16%) of third degree. All 25 patients showed negative scraping for the hyphae form after treatment (cure of first degree of hyphae was 25(100%).

##### Group D (topical ketoconazole, 2% shampoo)

Cure of the yeast form was found to be 3(12%) of first degree, 9(36%) of second degree and 13(52%) of third degree.

Cure of the hyphae form was found to be 21(84%) of first degree and 4(16%) of second degree.

#### B- Comparison of four groups.

##### 1- According to the yeast form

Using chi-square test showed significant differences ( $p < 0.05$ ) of four groups after treatment.

While the greater cure of second degree was in group B, followed by group C, then group D. But the most cure of third degree was in group D, so group D was the least cure.

##### 2- According to the hyphae form

Table (4.2) shows the comparison of cure of four groups for hyphae. Using chi-square test showed significant differences ( $p < 0.05$ ) among four groups after treatment.

### Side Effects of Ketoconazole

#### Clinical reported side effects

##### A- Results of four groups

##### Group A (oral ketoconazole, 200 mg)

Clinically in this study there was no side effect reported in patients who received 200mg of ketoconazole, once daily for two weeks.

##### Group B (oral ketoconazole, 400mg)

Patients who received 400mg once /week for two weeks reported only one patient suffering from headache.

##### Group C (Topical 2% ketoconazole cream)

No side effect was reported in patients who received 2% ketoconazole cream, twice daily for two weeks.

##### Group D (Topical 2% ketoconazole shampoo)

In this group only one case reported rash and burning sensation. This patient did not follow the instructions given to him to bath after topical application of the shampoo.

## LABORATORY EXAMINATION

### EFFECT ON THE LIVER

Determination of the side effect of ketoconazole on the liver by liver function tests such as TSB, enzymes (SGPT and ALP) and albumin of patients in the four groups.

#### A- Results of four groups

Group A (oral ketoconazole, 200mg)

Table (4.3) shows mean  $\pm$  SEM of liver function tests before and after treatment in patients who received oral ketoconazole 200mg/day for two weeks.

Group B (oral ketoconazole, 400mg)

Table (4.3) shows mean  $\pm$  SEM of liver function tests before and after treatment in patients who received oral ketoconazole 400mg as a single dose for two weeks. T-test shows significant differences ( $p < 0.05$ ) for TSB, SGPT, ALP (i.e: increase in the TSB, SGPT and ALP after treatment) and non significant differences for albumin ( $p > 0.05$ ).

Group C (topical 2% ketoconazole cream)

Table (4.3) shows mean  $\pm$  SEM of liver function tests before and after treatment in patients who received 2% cream, twice daily for two weeks.

Group D (topical 2% ketoconazole shampoo)

Table (4.3) shows mean  $\pm$  SEM of liver function tests before and after treatment in patients who received 2% shampoo for 3 days.

B- Mycological Investigations

C- Isolation of *Malassesia furfur*

Cultures of scales from the 100 patients showed that 89(89%) of the cases were given positive cultures while 11(11%) of the cases were given negative cultures.

D- In vitro Antifungal Susceptibility Test

The MIC of drug was used to determine in vitro the activity of ketoconazole against 40 clinical isolates of *M. furfur* by broth dilution method.

In this study all isolates of *M. furfur* were sensitive to ketoconazole. (table 4.4) shows inoculum size, drug concentrations, range of MIC, MIC<sub>50</sub> and MIC<sub>90</sub>. Table 4.5 shows that ketoconazole acts as a completely fungicidal action (MFC) for 11 isolates and

incompletely cidal (MFC) for 8 isolates and fungistatic action (MIC) for 21 isolates.

## DISCUSSION

Pityriasis versicolor is an old, common chronic fungal infection of the skin<sup>11</sup>. It affected the stratum corneum as opportunistic infection by *M. furfur* (*Pityrosporum orbiculare*)<sup>12</sup>.

Previous studies in Iraqi people had shown that more than 90% of the healthy individuals carry *M. furfur* (*P. orbiculare*) on their bodies as a normal flora of the skin<sup>13</sup> (Al-Rubaie, 1991). This explain the increase incidence of the disease in Iraqi people. PV present with scaly lesions and variable in colour causes great cosmetic problems especially when found on the exposed parts of the body.

Clinical cure showed disappearance of hyperpigmented lesions while hypopigmented disappeared within few weeks after treatment. This is agreement with the fact that the fungus may filter the rays of sun and interfere with the normal tanning<sup>1</sup> and the metabolites of *M. furfur* can cause depigmentation by inhibiting tyrosinase<sup>14</sup>.

Cure was confirmed by wood's light examination 95% of cases. While direct microscopic examination with (10% KOH) showed predominantly yeast than hyphae form and cure of first degree was 64%, 44%, 40%, 12% in regimens A, B, C, D respectively for yeast and for hyphae was 100%, 100%, 100%, 84% in regimens A, B, C, D respectively.

Side effects of ketoconazole were confirmed by liver function test which showed significantly increased TSB, SGPT, ALP and decreased albumin in the four groups after treatment. the comparison of four groups showed significantly more elevation in TSB in group A than C,D and significantly more elevation in SGPT in A than B and less elevation in C, D. while significantly more decreasing in albumin in A

than B. C,D were less decreasing than A but more decreasing than B. These results of a symptomatic transient of liver function tests disturbances could be explained by hepatocellular pattern of damage and sometimes cholestasis and elevation of aminotransferase activity in plasma are common and these values revert to the normal spontaneously while symptomatic drug inducing liver injury is rare<sup>15</sup>.

The antifungal susceptibility pattern of fungus revealed that ketoconazole was highly effective against *M. furfur* with the MIC ranging from 0.03-0.5 µg / ml. In this study no strains were resistant to ketoconazole. Drug acts as fungicidal in 19 (47.5%) of isolates and fungistatic in 21 (52.5%) of isolates. So this result showed relatively small differences between inhibitory and fungicidal values for ketoconazole. These results could be explained that if drug treatment may be failure that was not related to resistant of organism to ketoconazole but to the organism as a part of normal flora of skin, presence of predisposing factors and inadequate dose and duration of treatment.

The study concluded that, in the present work ketoconazole was highly effective in vivo and in vitro and no strains were resistant to ketoconazole. Also, in the clinical trial reported the great cure in group A (200mg) followed by group B (400mg), then group C (2% cream) and group D (2% shampoo) was the less cure. Further, this study reported great side effect in group A followed by C, D then B. Finally, the present study helps to throw more light on the MIC of drugs against fungi. This is useful to open the fields of research by using the same procedure for other drugs and other fungi. This is given the important value in the clinic.

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**Table (4.2) :Comparison of results of direct microscopic examination (hyphal form) after treatment of four groups.**

Results after treatment (cure)	Group A *KTC 200mg		Group B KTC 400mg		Group C KTC cream		Group D KTC shampoo		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
(1 <sup>st</sup> degree)-ve	25	100	25	100	25	100	21	84	96	96
(2 <sup>nd</sup> degree)few	0		0		0		4	16	4	4
<b>Total</b>	<b>25(100%)</b>		<b>25(100%)</b>		<b>25(100%)</b>		<b>25(100%)</b>		<b>100(100%)</b>	

\*KTC: ketoconazole , \*1 st. degree of cure = (0 organism) , \*2 nd. degree of cure = few organisms, -ve =0 organism, Few = (not more than 10 organisms), Chi-square (X<sup>2</sup>) =12.50, p=0.006< 0.05

**Table (4.3): Results of liver function test before and after treatment of four groups.**

Group	TSB	SGPT	ALP	Albumin
	mean±*SEM mg/dl	mean±SEM units/ml	mean±SEM units/L	mean±SEM g/dl
<b>A</b>				
Before. *T	0.80±0.04	11.24±1.15	48.85±2.17	2.88±0.05
After. T	0.97±0.04	16.08±1.22	53.11±2.11	2.36±0.05
Significance	P=0.0005< 0.05	P=0.0005<0.05	P=0.001<0.05	P=0.0005<0.05
<b>B</b>				
Before. T	0.70±0.03	6.96±1.13	49.91±2.03	2.52±0.13
After. T	0.89±0.03	10.36±1.07	53.00±2.34	2.34±0.05
Significance	P=0.0005<0.05	P=0.0005<0.05	P=0.0005<0.05	*NS
<b>C</b>				
Before. T	0.67±0.04	7.28±1.15	48.51±1.79	2.89±0.07
After. T	0.76±0.05	9.48±1.22	52.30±2.08	2.51±0.07
Significance	P=0.008<0.05	P=0.006<0.05	P=0.0005<0.05	P=0.0005<0.05
<b>D</b>				
Before. T	0.70±0.04	8.90±1.16	51.07±2.14	2.99 ± 0.11
After. T	0.80±0.05	11.00±1.55	53.48±2.67	2.62 ± 0.07
Significance	P=0.001<0.05	P=0.0005<0.05	P=0.001<0.05	P=0.0005<0.05

\*T: treatment , \*SEM: standard error of mean, \*NS: non significant differences

**Table (4.4): Results of MIC for yeast of *M. furfur***

No. of isolate	Inoculum size	Range of drug concentration	* MIC range	mean±SEM	MIC <sub>50</sub>	MIC <sub>90</sub>
40	1-5X10 <sup>4</sup> Cfu/ml	0.03-16 µg/ml	0.03 -0.5 µg/ml	0.11 ± 0.01 µg/ml	0.06 µg/ml	0.25 µg/ml

\*MIC: minimum inhibitory concentration

Table (4.5): MIC value, fungicidal and fungistatic drug concentration of 40 isolates

Isolate No.	MIC µg/ml	Colonies number	Fungistatic *( MIC )	Fungicidal *(MFC)	
				Complete	Incomplete
23	0.06	0		0.06	
24	0.06	8	0.06		
27	0.06	6	0.06		
31	0.06	3			0.06
35	0.06	0		0.06	
41	0.06	7	0.06		
42	0.06	10	0.06		
44	0.5	6	0.5		
50	0.03	0		0.03	
52	0.06	0		0.06	
57	0.03	2			0.03
58	0.06	10	0.06		
59	0.06	6	0.06		
60	0.06	0		0.06	
63	0.06	3			0.06
64	0.06	0		0.06	
65	0.03	7	0.03		
68	0.06	2			0.06
70	0.06	6	0.06		
72	0.5	0		0.5	
75	0.06	8	0.06		
76	0.25	7	0.25		
77	0.03	11	0.03		
78	0.06	9	0.06		
81	0.06	2			0.06
82	0.125	3			0.125
84	0.03	8	0.03		
89	0.06	3			0.06
92	0.03	0		0.03	
93	0.25	7	0.25		
96	0.125	9	0.125		
97	0.06	6	0.06		
101	0.06	5	0.06		
103	0.125	2			0.125
112	0.5	0		0.5	
121	0.25	0		0.25	
124	0.125	0		0.125	
131	0.25	8	0.25		
132	0.06	8	0.06		
136	0.06	7	0.06		

## دراسة مقارنة لاستخدام KETOCONAZOLE عن الطريق الفموي والسطحي في علاج PITYRIASIS VERSICOLOR

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### الخلاصة

الهدف الأول من الدراسة هو دراسة مقارنة للكيتوكونازول الفموي والسطحي في علاج Pityriasis versicolor والهدف لتحديد فعالية الدواء ضد الفطر باختبار التركيز المثبط الأدنى. شملت الدراسة السريرية 178 مريض . كل مريض فحص سريريا ومختبريا قبل وبعد العلاج. تم تأكيد الشفاء بعد العلاج باختبار ضوء الخشب 95% من الحالات كانت بدون أشعاع بينما الاختبار ألمجهري المباشر اظهر شفاء من الدرجة الأولى 64%، 44%، 40%، 12% في المجموعة A, B, C, D على التوالي للخمائر أما للغزول فكانت النسب 100%، 100%، 84% للمجاميع أعلاه على التوالي. فيما يتعلق باختبار فحص الحساسية أظهرت نتائج الدراسة بان الكيتو كونازول كان موثر بشكل عالي ضد 40 عزلة من الفطر مع قيم ادني تراكيز مثبطة دنيا تراوحت من 0.03-0.5 وكان MIC50 يساوي 0.06 و MIC90 يساوي 0.25 مايكرو غرام/مل. اظهر الدواء تركيزا قاتلا في 19 (47.5 %) من العزلات وتركيزا مثبطا في ( 52.5 %) 21 من العزلات. تستنتج الدراسة بان الدواء كان موثرا بشكل عالي في جسم الإنسان والمختبر كما واطهر التقييم السريري بان الشفاء الأكبر كان في المجموعة A (200مغم) ثم تليه المجموعة B (400 مغم) ثم المجموعة C (2% دهن) والمجموعة D (2% شامبو) كان الأقل شفاء. كذلك التأثير الجانبي الأكبر في المجموعة A ثم تبعته المجموعة C ثم D ثم B.