STUDY THE EFFECT OF NIZORAL ON SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN MALE RABBITS

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ABSTRACT

The present study aimed to determine the potential toxic effects of Nizoral through oral administration on some of blood and biochemical parameters of laboratory rabbits .

Eighteen of local normal rabbits were used in the present study . The animal divided randomly to three group (six animal in each) .Group one (control) the animal treated normal saline .Group two the animals chronically feed by stomach tube Nizoral 20 mg/kg/BW daily for 30 days. Group three the animals chronically feed by stomach tube Nizoral 40 mg/kg/BW daily for 30 days. The investigation included body weight gains (BW) , some blood parameters like red blood cells count (RBC) hemoglobin concentration(Hb), packed cell volume (PCV) and total white blood cell count(WBC) . Biochemical parameters included total serum alan in aminotransferase (ALT), aspartate aminotransferase(AST),and hormones testosterone concentration ,and semen characteristics .The results showed reduced in BW. R.B.C count ,Hb concentrations PCV value were reduced gradually but not reach a significantly . Total W.B.C count lymphocyte and monocyte appeared a significant decrease($p \leq 0.05$) ,but neutrophils showed a significant increase ($p \leq 0.05$).The biochemical study refers a significant increase ($p \leq 0.05$) in ALT,AST and a significant decrease($p \leq 0.05$) in hormones testosterone concentration .The testes and epididymis appeared absences of sperms .

INTRODUCTION

Nizoral is antifungal agent, Its synthesis has been reported by (1,2). Nizoral is a synthetic broad-spectrum antifungal agent. It is highly lipophilic compound, so it is highly concentrated in fatty tissue. The drug is available as oral tablets, cream, and shampo formulations, it is protein bound 84%-99%, metabolized in liver with biphasic half life. The initial phase is 2 hours and the mean peak plasma levels are reached within 1 to 2 hours of following oral taken with a meal. The drug is excreted via biliary and renal routes of excretion(3).

Accumulation of Nizoral in fatty tissues . It less toxic and more effective after oral administration, nizoral is best absorbed at highly acidic levels in the gastrointestinal tract and more rapid absorption in rats and guinea-pigs than in rabbits and dogs, metabolized in the liver and is converted into many one of the group that affects cytochrome p-450 (CYPs) . The mechanism of action Nizoral works principally by the inhibition of cytochrome P-450, (4).It has been found that administration of high doses of Nizoral to the human lead to weight gain. Study the effect of Nizoral at a dose of 80 mg/kg/day administered on Wister rats caused significant decrease in food consumption as well as to the appearance of clinical signs of toxicity ,diarrhea characterized by a decrease in weight gain, decrease in food and water consumption. (5)

MATERIAL AND METHOD

The experiment was conducted at the animal house of Veterinary Medicine College – University of Basra. Where 18 males rabbits age (8) week old and average body weight between (2 – 2.5)Kg were used. The animals were accommodated in the same laboratory condition by keeping them in special cages. The experiment conditions were unified for all animals where the room temperature was set between 20-25C by the use of air conditioner, and the light period was 12 hours daily, by the use of two fluorescent lamps ,and the humidity rate was about 50% food and water were provided daily .The animals

of the experiment was divided randomly into three groups with 6 male rabbits in each, as follows:

Group1(control):administered orally 0.9% normal saline (N.S) daily for one month.

Group 2: administered orally 20mg/kg of Nizoral daily for one month.

Group 3 : administered orally 40mg/kg of Nizoral daily for one month.

Collection of blood samples.

Blood sample (5 ml) were collected from heart puncher . Blood sample(2 ml) collected from each animal were stored in plastic sample test tube containing ethylene diamine tetra acetic acid (EDTA) anticoagulant for hematological studies which done directly after collection .However another portion (3 ml) of blood was deposited in to tube without anticoagulant and allowed to clot at room temperature . The blood samples were centrifuged at (5000 rpm) for 30 minutes and serum sample were stored in polyethylen tubes at (– 20c) until used for biochemical analysis .

Hematological study

The hematological tests were done in the laboratory veterinary college by using Hematology auto analyzer (Huma Counts 5) made in Germany company serial no.160247 . the instrument can measures and calculates 22 different parameters .The Hematology auto analyzer containing four solution(HC5 – BASOLYSE containing cyanide free lyse reagent ,HC-LYSECF containing cyanide free lyse reagent ,HC5 – EOLYSE containing cyanide free lyse reagent and HC- Cleaner cleaning solution used to clean fluidics system) and the instrument have a printer mechanic inside with thermal paper . The hematological parameter estimated by this instrument were (RBC, WBC. DWBC ,Hb and PCV).

Biochemical test

The biochemical tests were done in the laboratory of veterinary college by using chemistry auto analyzer made in Germany by human star company serial no.20628 ,the machine has 54 wells which numbered from 1 to 54 , The serum samples deposited in

each specific wells . The reagent was put in a special container beside the wells. The serum biochemical parameters estimated by this instrument were AST and ALT.

Estimation of Testosterone Hormone

Determination of total Testosterone concentration in serum by microplate enzyme immunoassay , kit was used (monobind Inc .lake forest CA 92630, USA).

Semen examination

The individual motility of the Sperms was measured depending upon the graduation basis suggested by(6). The dead and live spermatozoa were counterd according to (7) .

Statistical analysis:

The result of present study were analysis by univarate analysis of variance, the data were expressed as mean -+ standard deviation (mean ±SD.) least significant different test (LSD) was used to test the difference between means (group) by using statistical program for social science SPSS. $P<0.05$ was considered significant .

RESULT

The effect of 20- 40 mg/kg/ Bw daily Nizoral for 30 days on the body weight gains are shown in the table (1) .The result shows a significant increase ($p\leq0.05$) in the body weight gains with advanced period of treatment in the control group . While a gradual significant decrease($p\leq0.05$) in the body weight gains were recorded in the 20, 40

mg/kg BW Nizoral treated groups with advanced period of treatment compared with control .

Table (1) The effect of Nizoral 20-40 mg/kg/bw for 30 days on Body weight.

Parameters	Treatment	D0	D10	D20	D30
B.W Kg%	Control	2.35 ± 0.23 a	2.83 ± 0.16 a	3.51 ± 0.14 a	3.96 ± 0.11 a
	KCZ 20 mg/kg	2.40 ± 0.10 a	2.51 ± 0.04 a	2.21 ± 0.09 b	2.22 ± 0.13 b
	KCZ 40 mg/kg	2.55 ± 0.17 a	2.80 ± 0.05 a	1.97 ± 0.18 c	1.43 ± 0.21 c

(Mean ± SD) (n= 6).
The smal letters refer to significant differences at ($p \leq 0.05$) between groups .

The RBC count , Hb concentration and PCV values in the table (2) clearly indicate that the means of RBCs count were reduced gradually but not reach a significantly ($p \leq 0.05$) in 20 and 30 days of treatment by 20 mg/kg BW and 40 mg/kg BW of Nizoral treated group respectively compared with control group . On the other hand Hb concentration ,PCV value also appeared decrease gradually but not reach a significantly ($p \leq 0.05$) in 20 and 30 days of treatment by 20 mg/kg BW and 40 mg/kg BW Nizoral treated group when compared with control group.

Table (2) The effect of Nizoral for 30 days on RBC count ,Hb concentration and PCV value . (Mean ± SD) (n= 6)

Parameters	Treatment	D0	D10	D20	D30
RBC (Cell x 10^6)	Control	5.37 ± 1.21 a	5.41 ± 1.26 a	5.53 ± 1.14 a	5.96 ± 1.31 a
	KCZ 20 mg/kg	5.20 ± 2.02 a	5.83 ± 1.78 a	5.10 ± 1.55 a	6.01 ± 2.33 a
	KCZ 40 mg/kg	5.72 ± 1.91 a	5.24 ± 1.26 a	5.13 ± 1.11 a	5.16 ± 1.94 a
Hb (gm /dl)	Control	10.72 ± 1.17 a	10.80 ± 0.98 a	11.17 ± 0.68 a	10.13 ± 0.21 a
	KCZ 20 mg/kg	11.10 ± 0.14 a	10.45 ± 0.05 a	10.53 ± 0.20 a	10.22 ± 0.51 a
	KCZ 40 mg/kg	11.16 ± 0.31 a	10.74 ± 0.32 a	10.11 ± 0.19 a	10.04 ± 0.33 a
PCV %	Control	36.72 ± 1.27 a	37.80 ± 0.98 a	35.17 ± 0.48 a	37.13 ± 0.21 a
	KCZ 20 mg/kg	37.11 ± 0.17 a	35.16 ± 0.55 a	34.56 ± 0.15 a	34.22 ± 0.51 a
	KCZ 40 mg/kg	36.16 ± 1.44 a	35.74 ± 0.45 a	35.11 ± 0.18 a	33.64 ± 0.21 a

The small letters refer to significant differences at ($p \leq 0.05$) between groups

Table (3) show that the total WBC count decrease significantly ($p \leq 0.05$)with the advanced period of treatment in all treated groups compared with control . The lymphocytes percentage was significantly decrease ($p \leq 0.05$) in the 30 days of treatment in 20 mg/kg BW treated group and in (20 and 30 days) of treatment in 40 mg/kg BW Nizoral treated groups compare with control .

The monocyte percentage revealed decrease a significantly ($p \leq 0.05$) with the advanced period of treatment only in 40 mg/kg BW Nizoral compared with control .

The same table showed also Nizoral caused a significant increase($p \leq 0.05$) in the percentage of the neutrophils number especially in 20 and 30 days of treatment in the 40 mg/kg Nizoral treated group compared with control , but there was no significant different in eosinophils and basophils .

Table (3) The effect of Nizoral for 30 days on total WBC count and differential WBC %. . (Mean ± SD) (n= 6).

Parameters	Treatment	D0	D10	D20	D30
WBC (1 x $10^{3)}$	Control	34.60 ± 2.10 a	31.97 ± 3.34 a	38.94 ± 3.12 a	37.29 ± 1.87 a
	Nizoral 20 mg/kg	32.61 ±2.64 a	29.77 ±3.63 a	24.79 ± 2.10 b	21.98 ± 2.08 b
	Nizoral 40 mg/kg	39.12±1.66 a	31.16 ±5.32 b	23.36±3.30 c	22.50 ± 2.54 c
Lymphocyte (%)	Control	46.44 ± 2.42 a	45.02 ± 2.56 a	48.51 ± 2.18 a	47.09 ± 2.56 a
	Nizoral 20 mg/kg	44.60 ± 2.31 a	45.22 ± 2.22 a	45.03 ± 2.03 a	27.00 ± 2.24 b
	Nizoral 40 mg/kg	4٥.10 ± 2.17 a	45.20± 2.00 a	30.11 ± 2.12 b	25.10 ± 2.19 c
Monocyte (%)	Control	9.13± 2.15 a	9.33±2.47 a	8.35±2.31 b	9.01±2.90 a
	Nizoral 20 mg/kg	8.13+2.51 a	8.75+2.11 a	7.16+2.33 a	5.08 +3.22 b
	Nizoral 40 mg/kg	8.43± 2.41 a	9.21±1.80 a	6.13 ± 3.07 b	4.81±2.86 c
Eosinophils (%)	Control	5.03±1.45 a	5.47±1.18 a	5.07±1.00 a	4.03±1.40 a
	Nizoral 20 mg/kg	5.33±10.60 a	6.83 ± 2.53 a	4.53 ± 2.10 a	4.23± 2.19 a
	Nizoral 40 mg/kg	5.11± 2.09 a	5.73 ±2.22 a	4.21±3.90 a	4.15± 2.85 a
Neutrophils (%)	Control	33.10 ±2.86 a	31.25 ±3.12 b	31.30 ±1.94 c	32.68 ±2.21 c
	Nizoral 20 mg/kg	32.45±17.72 a	33.00±23.11 a	41.12±18.32 b	62.9± 21.93 b
	Nizoral 40 mg/kg	32.72 ±22.03 a	31.82 ±23.08 b	46.01±17.35 a	78.81±12.76 a
Basophils (%)	Control	01.00 ± 0.00 a	00.01 ± 0.00 a	01.00 ± 0.00 a	01.00 ± 0.00 a
	Nizoral 20 mg/kg	01.00 ± 0.00 a	00.00 ± 0.00 a	00.00 ± 0.00 a	02.00 ± 0.00 a
	Nizoral 40 mg/kg	00.02 ± 0.00 a	00.00 ± 0.00 a	01.02 ± 0.00 a	01.01 ± 0.00 a

The small letters refer to significant differences at ($p \leq 0.05$) between groups

The effects of Nizoral treatment for 30 days in intact male rabbits on the serum AST , ALT and hormone testosterone concentration are presented in the table (4) . A gradual significant increase($p \leq 0.05$) in AST concentration was observed in 20 and 40 mg/kg/ BW Nizoral treated group started from day 10 to day 30 of treatment compared

with control. The same table also revealed a significant increase($p \leq 0.05$) in ALT concentration in the same treatment and period.

On other hand the concentration of testosterone hormone appeared a significantly decreased ($p \leq 0.05$) from 20 and 30 days of treatment in 20 mg /kg Bw Nizoral treated groups and in days 10. 20 and 30 in 40 mg /kg Bw Nizoral compared with control . There was no sperms was seen in both doses of Nizoral treated groups when compared with control group table (5).

Table (4) The effect of Nizoral for 30 days on AST, ALT and Testosterone hormones concentration . (Mean ± SD) (n= 6).

Parameters	Treatment	D0	D10	D20	D30
ALT U/I	Control	35.01 ± 2.14 a	33.01 ± 2.11 c	35.01 ± 2.36 c	34.01 ± 2.64 c
	Nizoral 20 mg/kg	33.43 ± 2.30 c	35.01 ± 2.73 b	38.12 ± 2.54 b	45.64 ± 2.29 b
	Nizoral 40 mg/kg	35.71 ± 2.11 d	39.01 ± 2.46 a	43.01 ± 0.21 a	48.54 ± 0.44 a
AST U/I	Control	25.61 ± 2.64 a	24.03 ± 2.12 a	24.24 ± 2.01 a	24.11 ± 2.48 c
	Nizoral 20 mg/kg	24.45 ± 2.42 a	23.73 ± 2.25 b	26.24 ± 2.27 b	33.56 ± 2.95 b
	Nizoral 40 mg/kg	25.38 ± 2.05 a	26.93 ± 2.62 c	34.24 ± 2.76 b	38.11 ± 2.14 a
Testosterone ng /L	Control	21.01 ± 1.44 a	21.93 ± 1.12 a	22.24 ± 1.21 a	22.11 ± 1.48 a
	Nizoral 20 mg/kg	22.18 ± 1.24 a	21.73 ± 1.42 a	17.01 ± 1.74 b	15.10 ± 1.16 b
	Nizoral 40 mg/kg	23.85 ± 1.54 a	20.13 ± 1.64 a	15.14 ± 1.14 c	11.12 ± 1.11 c

The small letters refer to significant differences at ($p \leq 0.05$) between groups

Table (5) The effect of Nizoral for 30 days treatments on Semen characteristics . (Mean ± SD) (n= 6).

Treatments	Semen characteristics			
	individual motility %	Abnormal sperms %	Dead sperms%	Sperms concentration x^6
Control	79.30± 5.63 a	12.08 ± 4.89 a	22.71± 4.11a	198.1 ± 34.19 a
Nizoral 20 mg/kg	0.00 b	0.00 b	0.00 b	0.00 b
Nizoral 40 mg/kg	0.00 b	0.00 b	0.00 b	0.00 b

The small letters refer to significant differences at ($p \leq 0.05$) between groups.

DISCUSSION

Nizoral is the first cost-effective broad spectrum, oral anti-fungal agent in a series of Azole derivatives, it is successfully in the treatment of fungal infection (8), but it is associated with some hepatic damage.

The results in the present study revealed significant decrease in body weight when the animal treated with ٢٠ and ٤0 mg /kg day Nizoral starting from day ٢0 to ٣0 respectively when compared with control , Table (1) .This finding is in agreement with (5) who found that effect of Nizoral at a dose of 80 mg/kg/day on Wistar rats caused a significant decrease in food consumption as well as the appearance of clinical signs of toxicity ,diarrhea characterized by a decrease in weight gain, decrease in food intake. .However it is finding disagreement with previous study(4) in which that administration of high doses Nizoral to the human lead to weight gain, also administration of Nizoral caused hyper phage in rats(9).

There was no significant difference in total RBC count , Hb and PCV concentration in blood of male rabbits treated with 20 and 40 mg/kg BW Nizoral from day 10 -30 of treatment compared with control group table (2) .

The mean values of total WBCs count and differential white blood cell count are represent in the Table (3). The results of WBCs count , lymphocyte and monocyte percentage obtained in the present study are decreased significantly in all treatment compared with control .Chronic oral administration of Nizoral caused significant reduced in the lymphocyte proliferation and monocyte count of mice 10-18%, these change could be due to change in p-glycoprotein function (10)

The result in table (4) appeared increased in ALT and AST concentration started from day 20 to 30 of treatment when the rabbits treated 20 and 40 mg/kg BW Nizoral , The mechanism of injury was suspected as a reaction of Ketoconazole (11), at the beginning toxic metabolites damage the smooth endoplasmic reticulum them produces further injury to mitochondria and plasma membrane. Those events lead to cloudy

swelling, ballooning degeneration and necrosis of hepatocytes (12). Many retrospective studies on liver show use of different doses of Ketoconazole for induction of toxicity such as 40 and 90 mg/kg of body weight (13). The result agree with (12) who noted that administration of Nizoral in rabbit 40, 80, and 160 mg/kg, resulted in a significantly increase in serum ALT after 36 h may be due the ALT is a hepatocyte-specific enzyme , So the increase in its serum activity is principally due to hepatic parenchymal disease, also Nizoral caused hepatotoxicity characterized by the development of severe hepatitis with marked centro lobular necrosis. (14).

There was a significant decrease in hormone testosterone concentration in serum compared with control group . Nizoral reduces adrenal steroid production though inhibition of multiple steroidogenic enzymes, which effects on 11β-hydroxylase, 17α-hydroxylase and 18-hydroxylase (15).Ketoconazole also caused that inhibits adrenal and testicular steroids reversibly and blockade of testosterone synthesis by inhibitory activity against enzymes involved in the synthesis of the activity 17, 20-lyase of the enzyme CYP17, which is involved in the synthesis of testosterone, ketoconazole may have more generalized inhibitory effects on steroidogenic tissue function (16 and17).It has been

found that administration of ketoconazole to rats caused testicular damage table (18) . A single oral dose (300 mg/ kg) of KCZ induced reversible immobilization of rat epididymal spermatozoa at 8–24 h after dosing, these findings suggest that KCZ gains access to the post-testicular sex organs and affects the mature spermatozoa there in much more readily than it affects testicular spermatogenesis. (19) .

دراسة تاثير عقار النيزورال على بعض المعايير الفسلجية والكيميائية على ذكور الارانب

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الخلاصة

صممت الدراسة لتقييم التاثيرات السمية لعقار Nizoral على بعض المعايير الدمية والبايوكيميائية في الارانب المحلية

استخدمت في هذه الدراسة ١٨ من الارانب المحلية الطبيعية والتي تم تقسيمها عشوائيا الى ثلاثة مجاميع . المجموعة الاولى (السيطرة) جرعت(٠.٥ مل) المحلول الفيسيولوجي ٠.٩% المجموعة الثانية جرعت الحيوانات عقار كيتوكينازول جرعة ٢٠ ملغم لكل كغم من وزن الجسم يوميا ولمدة ثلاثون يوم والمجموعة الثالثة جرعت الحيوانات عقار كيتوكينازول جرعة ٤٠ ملغم لكل كغم من وزن الجسم يوميا ولمدة ثلاثون يوم ، بعدها اجريت الاختبارات التالية ، معدل الزيادة الوزنية ، عد كريات الدم الحمراء وتركيز هيموغلوبين الدم وحجم الخلايا المضغوطة وعد خلايا الدم البيضاء والعد التفريقي لها اضافة الى اخذ اختبارات انزيمات الكبد وقياس تركيز هرمون التستسترون واختبارات السائل المنوي .

اظهرت نتائج الدراسة الحالية ان التغيرات الحاصلة في كل من عد كريات الدم الحمراء وتركيز هيموغلوبين الدم وعدد خلايا الدم البيضاء والخلايا اللمفاوية والاحادية النواة لم تصل الى حد المعنوية بينما اظهرت الخلايا المتعادلة ارتفاعا معنويا واضحا مقارنة مع مجموعة السيطرة . كذلك اظهرت النتائج ارتفاع معنوي في معدل تركيز انزيمات الكبد وانخفاض معنوي في تركيز هرمون التستسترون والذي ادى بالنتيجة الى انعدام وجود الخلايا النطفية في كل من الخصى والبربخ عند قياس خواص السائل المنوي .

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