Clinicopathological Studies on the Effect of Antifungal Drugs in Rabbits

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ABSTRACT

The present study was performed to evaluate the side-effects of itraconazole and nystatin, besides the ameliorating effect of propolis against the side-effects of such drugs using the clinicopathological changes.

Sixty healthy New Zealand male rabbits,(one month old and 500 gm body weight) were divided into 6 equal groups. Gp.1,was the control. Gps.2-6 were orally given itraconazole, nystatin, propolis ,combination of the half therapeutic dose of itraconazole and propolis and combination of the half therapeutic dose from nystatin and propolis,respectively daily for one month using stomach tube, then treatments were stopped for 7 days. Blood samples were collected after 15 (1st period) and 30 (2nd period)days from start of experiment and after 7 days (3rd period)after the end of treatment.

Gp. 2 showed increased serum ALT,AST and ALP activities ,besides the total, direct, indirect bilirubin, total lipids, cholesterol, triglycerides, urea, creatinine, calcium, inorganic phosphorus and potassium levels in addition to decreased serum albumin, globulin and sodium levels. Hemolytic anemia, neutropenia and eosinophilia were seen .Those changes were ameliorated in gp.5.

Gp.4 showed improvement of the previous parameters. Gps.3 & 4showed no changes when compared with the control. The side effects produced by antifungal drugs, need more than 7 days to regain the normal level.

INTRODUCTION

The prevalence of fungal infections has increased significantly during the past decade. This increase is due to the greater use of broad spectrum antibiotics, immunosuppressive agents and acquired immunodeficiency syndrome *(1).*

Resistance to antimicrobial agents has become increased and pressing global problem *(2).*Structural modification of antimicrobial drugs to which resistance has developed was considered an effective means of extending the life span of antifungal agents such as the azoles *(3).*

Azoles antifungal agents have added greatly to the treatment of fungal infections *(4).* Itraconazole a triazole offers a wide antifungal spectrum and few adverse effects. It is effective against Aspergillosis, candidal infections and dermatophytoses *(5, 6).*

Nystatin is the first discovered antifungal polyene antibiotic which has a wide therapeutic application for superficial mycoses of the skin and mucous membranes *(7).* Natural products have been particularly a rich source of anti-infective agents *(8).*

Propolis, a natural product of honey bee, has been used for thousands of years in folk medicine *(9).*It has attracted much attention in recent years as a useful or potential substance used in medicine *(10).*

The aim of the present work was to elucidate the biochemical and hematological picture associated with itraconazole and nystatin antimycotic therapy. Moreover the efficacy of propolis for potentiating immunity and ameliorating the side effects of the two used antimycotic drugs.

MATERIALS AND METHODS

MATERIALS

1-Experimental animals

Sixty healthy male white New Zealand rabbits (500 gm body weight and 30 days old) were obtained from the Animal Farm, Faculty of Veterinary Medicine, Zagazig University. The animals were kept under hygienic conditions, in metal cages, fed on balanced ration and water ad-libitum.

2-Preparation of the used drugs

The recommended dose of itraconazole (itracon) is 100-200 mg daily (therapeutic dose of human) *(11).*Consequently, the therapeutic dose for rabbit is 4.65-9.3 mg/kg B.wt *(12).* The recommended dose of nystatin (mycostatin) is 15ml daily (therapeutic dose of human) *(13).* Consequently, the therapeutic dose for rabbit is 1 ml daily *(12).* The recommended dose of propolis is 1.4 mg/kg B.wt (therapeutic dose of human) *(14).* Consequently, the therapeutic dose for rabbit is 4.6 mg/kg B.wt *(12).* The therapeutic dose of Itraconazole,Nystatin and propolis for rabbit was calculated according to Paget and Barnes*(12).*

METHODS

The experimental design is summarized in Table 1.

Table 1. Experimental design

Design	Gps.	No of rabbits	Oral treatments daily given for 1 month			Blood samples
			Itracon (9.3 mg/kg B.wt)	Mycostatin (1 ml)	Propolis (4.6 mg/kg B.wt)	
Control group	1	10	-	-	-	After 15 (1st period) and 30 (2nd period) days from drug administration then after 7 days (3rd period) from cessation of the drugs
Experimental groups	2	10	+	-	-	
	3	10	-	+	-	
	4	10	-	-	+	
	5	10	+*	-	+*	
	6	10	-	+*	+*	

* Half the therapeutic dose.

A- Sample collection

Blood samples were collected from the marginal ear vein . The 1st sample was five ml without anticoagulant in a sterile test tube for separation of serum for biochemical analysis .The 2nd sample was 1ml in clean Wasserman tubes containing disodium salt of ethylenediaminetetraacetic acid (EDTA) for hematological examination *(15).*

B-Biochemical studies

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities besides the bilirubin levels (total, direct and indirect), total protein, albumin, globulin, total lipids, cholesterol, triglycerides, urea, creatinine, calcium, inorganic phosphorus, magnesium, sodium and potassium levels were determined by using test kits of Diamond-Egypt,Bio-Diagnostic, Spinreact,Dialab and Elitech.

C-Hematological studies

1-Erythrogram

Erythrocytic count was carried out by using a hemocytometer and Gower's solution, hemoglobin (Hb) was determined by using cyanmethemoglobin. Packed cell volume (PCV) was determined by using microhematocrit tubes. MCV and MCHC were calculated *(16).*

2- Leukogram

The total leukocytic count was carried out by using a hemocytometer and Turkey's solution. Blood smears were prepared, fixed with absolute methyl alcohol (95%) and stained with Giemsa's stain for differential leukocytic counts and the absolute differential leukocytic counts were calculated *(16).*

D-Statistical analysis

The obtained data was analyzed using F-test *(17).*

RESULTS AND DISCUSSION

Table 2 shows marked increase in liver enzyme activities (ALT and AST) which were markedly pronounced in gp. 2 and moderate in gp. 5 . Hepatocyte injury was reflected by elevated serum activities of enzymes that leaked from hepatocytes .Necrosis in a tissue can produce high serum enzyme activity *(18).* Itraconazole hepatotoxicity could be attributed to production of toxic metabolites, mitochondrial

toxicosis and inhibition of mammalian sterol synthesis *(19).* After stopping drug administration for 7 days, the previous parameters values showed significant increase than control values but lower than those obtained after thirty days of drug administration. The rise in ALT and AST may need longer time to return to the control level *(20).* In rabbits , alkaline phosphatase is present in nearly all tissues .It is found in association with cell membranes and especially in intestinal epithelium ,renal tubules ,osteoblasts, liver and placenta *(21).*Significant increase in ALP activity was found in group 2 throughout the experimental period. This may be due to hepatotoxic reactions with arrested bile flow (Cholestatic Injury) *(22).* At the same time drug metabolites can interfere with the synthesis and secretion of bile, leading to cholestasis *(23).*

Gp. 4 and gp. 6 revealed insignificant changes in ALT and AST, when compared with gp. 1 this may be due to the absence of side-effects for nystatin and the hepatoprotective potential of propolis which maintains cellular glutathione hydrogenase(GSH)content *(24).* The decrease in serum ALP activity in gps. 4&6 may be due to fact that propolis stimulates bile output *(25).* Gp. 3 showed non- significant changes in above mentioned parameters as nystatin is not absorbed after oral use *(26).*

Table 2 shows hyperbilirubinemia with increased conjugated and unconjugated bilirubin levels, in gp. 2 throughout the experimental periods.This may be due to biliary obstruction leading to obstructive jaundice and raised serum bilirubin values *(27),* besides hemolytic jaundice induced by hemolytic anemia. Gp. 5 showed hyperbilirubinemia in the first experimental period, then values declined till the end of the experiment. This may be due to the use of itraconazole at half therapeutic dose in addition to propolis extract which enhanced the choleretic activity which suggests stimulating action of liver microsomal enzymes thus propolis extract administration has stimulatory effect on bile output *(25).* Gps. 4 & 6 showed a significant decrease or insignificant change in bilirubin level in different experimental periods. This may be due to antioxidant effects for aqueous extract of propolis *(28).* Gp. 3 showed non significant changes in these parameters due to the fact that nystatin is not absorbed after oral use as previously mentioned *(26).*

Table 3 shows hypoalbuminemia and hypoglobulinemia in gp. 2 till the end of the experiment. Hypoproteinemia could be attributed to hypoalbuminemia which occurs in hepatic disease and albumin represents mostly the largest component of plasma proteins .Hypoalbuminemia may be attributed to liver damage as the liver is considered to be the main organ responsible for synthesis of the majority of plasma proteins *(15).* Although most gamma globulins, functioning in the immune system, are synthesized in lymphoid tissue, several other types (α and ß) are synthesized in the liver. Hepatic insufficiency can result in decreased synthesis and therefore decreased serum concentrations of globulins *(18).* Gp. 5 showed non- significant change in protein profile throughout the experimental period. This may be due to the low itraconazole (half therapeutic dose) hepatocellular protection by propolis with a consequent improvement of the albumin and protein synthesis. Such improvement eventually induced the repair of damaged tissue (replacement of enzymes and structural component) by toxic reactions *(29).* Significant increase in protein profile was observed in gp. 4 during drug administration followed by non-significant increase after stopping administration. This may be due to anabolic effect of propolis which diminishes amino acid concentrations in blood. Such amino acids were consumed by protein- synthesis and immunological processes by inducing gamma globulin synthesis *(30, 31).* Gps. 3&6 showed non significant changes in these parameters due to the fact that nystatin is not absorbed after oral use *(26).*

Regarding to lipid profile, Table 3 shows significant increase in serum concentrations of total lipids including triglycerides and cholesterol throughout the experimental period in gp. 2. This increase was lowered after stopping drug administration. This may be due to decreased incorporation of triglycerides into fat depots, decreased hepatic degradation of cholesterol and increased hepatic production of

very low density lipoproteins.The increased concentration of these parameters often results in visible lipemia *(18)*. Gp. 5 showed non-significant change throughout the experimental period. This may be due to the use of half therapeutic dose of itraconazole in addition to protective effect of propolis by its several bioactive components which may counteract oxidative damage by neutralizing reactive oxidants, increasing the efficacy of endogenous antioxidants and modulating the cellular redox state(32). Gps. 4 & 6 showed either significant or non- significant decrease in lipid profile during the period of drug-administration. This may be due to the key proteins of lipogenesis and lipidolysis (peroxisome proliferator-activated receptor -α (PPAR-α)and sterol regulatory element binding protein -1,SREBP-1) and microsomal enzyme (3-hydroxy-3-methyleglutaryl coenzyme A ,HMG-COA reductase). Administration of propolis augmented PPAR-α protein and reduced SREBP-1 protein in the liver. Therefore the decreased triglycerides in plasma and liver by propolis may be due to the changes of these proteins. Propolis decreased cholesterol synthesis by decreasing hepatic HMG-COA reductase *(33)*.

Regarding the renal function tests, Table 4 shows a significant increase in urea and creatinine throughout experimental period in gp.2.This may be due to nephrotoxicosis which was mainly caused by nepherotoxins. Nepherotoxicosis is associated with direct toxic effect of itraconazole on the renal tubular epithelium and renal vasoconstriction *(34)*.Gp. 5 showed non significant increase in serum urea and creatinine throughout the experimental period. This may be related to use of itraconazole (half therapeutic dose) and the protective effect of propolis which reverse the toxic effect of itraconazole through action of caffeic acid phenthyl ester which causes a marked reduction in the extent of tubular damage. This may be attributed to its free radical scavenging activity *(35)*.Non significant changes in these parameters were observed in gps. 3,4 & 6 .

Serum electrolyte- levels, Table 4 showed hypercalcemia, hyperphosphatemia, hyponatremia and hyperkalemia in gp. 2 throughout the experimental period. This may be related to the fact that rabbits have high total blood calcium- level which can vary over a wider range than other species.Rabbits absorb calcium in proportion to its concentration in the gut, and the kidney eliminates the excess. Hypercalcemia is a consequence of renal disease in rabbits because of the inability of the kidney to eliminate the excess calcium. Hyperphosphatemia occurs as a result of impaired renal phosphorus excretion due to kidney disease.The kidney is the main organ involved in phosphorus balance. Hyponatremia is usually associated with polyuric renal failure when the kidney cannot concentrated urine and urine flow in the renal tubules at too fast rate which prevents the sodium potassium exchange. Hyperkalemia can be the result of impaired renal excretion of potassium due to kidney disease. Also, severe tissue damage can also cause hyperkalemia by dispersing potassium into extracellular space *(26,36)* . Gp.5 showed milder changes in electrolytes than gp.2.This may be related to using half therapeutic dose of Itraconazole which reduced its unfavorable side-effect, besides propolis protective effect *(32)*.Gp.4 maintained the values of serum calcium, phosphorus, sodium and potassium throughout the experimental period . This may be related to the fact that propolis is supposed to help absorption and utilization of various minerals due to its contents of organic acid derivatives which in turn improve the physiological functions by regulating the ion dependant enzymatic activities*(37)*.

Table 5 shows macrocytic hypochromic anemia in gp. 2 till the end of the experiment.This could be due to acute liver damage resulting from the administration of Itraconazole. Metabolic changes due to vitamin E deficiency may lead to lipid peroxidation and pyruvate kinase instability. The latter leads to adenosine triphosphate (ATP) reduction, resulting in hemolysis *(38)*. The survival period of red blood cells in uremic cases is shortened. Uremic toxicity causes enzymatic alterations of the glycolytic pathway *(39)*. Macrocytic

hypochromic anemia in gp.5 may be related to unfavorable effect of Itraconazole early in the experiment, but disappeared later on. This may be related to the protective effect of polyphenolics of propolis to the red blood cell membrane*(40)* and the role of propolis on antioxidant status of erythrocytes *(41,42)*. Gp. 4 showed non- significant increase in erythrocyte in addition to a significant increase in hemoglobin and PCV volume throughout periods of drug administration. This may be due to a direct stimulating action of propolis on hematopoietic bone marrow and enhancing their growth and differentiation into colony forming cells *(43)*. Propolis increases the digestive utilization of iron which might produce a higher level of hemoglobin regeneration *(37)*.

Concerning the results of leukogram Table 5 reveals neutropenia in gp.2,late in the experimental period. This may be related to suppressed proliferation of stem and progenitor cells of bone marrow which are supposed to be a major target of drug *(44)*.

Eosinophilia was observed throughout the experimental period in gps. 2 & 5 .This may be due to increased eosinophilic growth factors such as IL-5. Drug induced eosinophilia usually resolves with discontinuation of the offending agent *(45)*.Gp. 4 showed a significant increase in the total leukocytic count, neutrophilic and lymphocytic counts clearly late in the experimental period and still till the end of the experiment.This may be due to the fact that propolis induces proliferation of leukocyte precursors *(43)*. Eosinophilia which appeared early in the experiment may be due to some substances in propolis as chemical caffeic acids which have allergic properties *(46)*.

It could be concluded that

1. Itraconazole caused hepatic injury, nephropathy, hemolytic anemia and neutropenia associated with eosinophilia.
2. Nystatin caused no side effect.
3. Propolis improved hepatic and renal functions, erythrogram and leukogram.
4. Combination of Propolis with itraconazole by half therapeutic dose,for each reduced the biochemical and hematological changes.
5. The side effects produced by using antifungal drugs need more than 7 days in order to return to normal state.

It is recommended that

Using itraconazole together with propolis (half therapeutic dose of each) to minimize side effects of itraconazole. Also, using of propolis with nystatin (half therapeutic dose for each) broaden nystatin uses.

Table 2. Some liver function tests (mean values ±SE) in gps.1-6 at the end of the 1st ,2nd and 3rd experimental periods.

Periods	Parameters / Gps.	ALT (U/l)	AST (U/l)	ALP (U/l)	Total bilirubin (mg%)	Direct bilirubin (mg%)	Indirect bilirubin (mg%)
1st experimental period	Control Gp.(1)	53.40 cd ±1.69	84.81 c ±1.34	16.00 c ±1.70	0.56 c ±0.04	0.21 b ±0.03	0.35 c ±0.02
	Itraconazole Gp.(2)	69.56 a ±3.88	96.84 a ±1.17	25.00 a ±1.37	1.12 a ±0.02	0.37 a ±0.04	0.75 a ±0.03
	Nystatin Gp.(3)	50.40 cd ±2.34	82.14 c ±1.17	12.87 c ±0.93	0.49 cd ±0.01	0.20 b ±0.01	0.29 c ±0.01
	Propolis Gp.(4)	57.35 bc ±3.32	80.62 c ±0.98	10.62 d ±0.48	0.45 de ±0.04	0.28 ab ±0.05	0.17 d ±0.03
	Itra.+Propolis Gp.(5)	65.07 ab ±2.75	92.15 b ±0.98	20.00 b ±1.37	0.77 b ±0.05	0.34 a ±0.04	0.43 b ±0.04
	Nys.+Propolis Gp.(6)	48.51 d ±1.62	80.80 c ±0.97	10.00 d ±1.12	0.37 e ±0.02	0.22 b ±0.01	0.15 d ±0.02
	F-test	*	*	*	**	*	**
	LSD	7.96	4.24	3.57	0.10	0.09	0.07
2nd experimental period	Control Gp.(1)	51.40 cd ±0.93	76.95 c ±1.77	20.00 b ±1.25	0.52 c ±0.04	0.17 b ±0.03	0.35 b ±0.02
	Itraconazole Gp.(2)	107.53 a ±1.92	160.72 a ±1.34	35.62 a ±2.34	1.20 a ±0.05	0.54 a ±0.05	0.66 a ±0.03
	Nystatin Gp.(3)	51.00 cd ±0.84	81.14 c ±1.85	22.50 b ±2.30	0.58 bc ±0.01	0.24 b ±0.02	0.34 b ±0.005
	Propolis Gp.(4)	54.91 bc ±1.69	78.00 c ±1.28	12.50 d ±0.99	0.49 c ±0.07	0.23 b ±0.06	0.27 c ±0.02
	Itra.+Propolis Gp.(5)	57.56 b ±1.46	86.91 b ±1.93	14.37 d ±0.76	0.66 b ±0.04	0.28 b ±0.06	0.38 b ±0.03
	Nys.+Propolis Gp.(6)	50.40 d ±1.03	80.09 c ±1.05	15.87 c ±0.04	0.33 d ±0.01	0.17 b ±0.01	0.16 d ±0.01
	F-test	**	**	**	**	**	**
	LSD	4.00	4.59	3.68	0.13	0.13	0.07
3rd experimental period	Control Gp.(1)	47.19 bc ±1.55	77.48 b ±1.33	14.61 c ±1.44	0.54 bc ±0.03	0.25 c ± 0.03	0.29 ab ±0.02
	Itraconazole Gp.(2)	52.11 a ±1.43	90.04 a ±1.05	20.77 a ±0.94	0.82 a ±0.03	0.49 a ±0.03	0.33 a ±0.03
	Nystatin Gp.(3)	43.08 c ±1.77	77.47 b ±1.78	13.07 c ±0.94	0.51 bcd ±0.01	0.22 c ±0.03	0.29 ab ±0.02
	Propolis Gp.(4)	49.22 ab ±1.71	75.92 b ±1.84	11.63 c ±0.32	0.39 d ±0.03	0.22 c ±0.03	0.16 c ±0.007
	Itra.+Propolis Gp.(5)	44.69 bc ±0.91	75.38 b ±3.14	17.69 b ±1.54	0.58 b ±0.08	0.37 b ±0.04	0.21 bc ±0.06
	Nys.+Propolis Gp.(6)	44.46 bc ±2.26	74.34 b ±1.78	8.46 d ± 0.35	0.43 cd ±0.04	0.25 c ±0.05	0.18 c ±0.02
	F-test	*	*	*	**	**	*
	LSD	4.84	5.64	3.02	0.12	0.10	0.08

Means in the same column and in same period followed by different letters are statistically significant and the highest value is represented with the letter a. LSD: Least significant difference.
*: Significant at 0.05 probability. **: Highly significant at 0.01 probability

Table 3. Some biochemical parameters (mean values±SE) in gps. 1-6 at the end of the 1st ,2nd and 3rd experimental periods.

Periods	Parameters / Gps.	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Total lipids (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
1st experimental period	Control Gp.(1)	5.67 b ±0.08	4.11 ab ±0.07	1.56 b ±0.06	203.60 b ±3.59	34.67 b ±2.18	39.99 bc ±3.61
	Itraconazole Gp.(2)	3.94 c ±0.11	2.76 c ±0.07	1.18 c ±0.09	311.80 a ±5.30	44.44 a ±2.43	70.85 a ±1.40
	Nystatin Gp.(3)	5.63 b ±0.11	3.96 ab ±0.08	1.67 b ±0.04	193.00 bc ±2.77	30.22 b ±1.66	34.28 cd ±1.81
	Propolis Gp.(4)	6.75 a ±0.21	4.18 a ±0.11	2.57 a ±0.11	185.80 c ±3.68	32.89 b ±3.33	32.00 d ±1.40
	Itra.+Propolis Gp.(5)	5.55 b ±0.12	3.79 b ±0.09	1.76 b ±0.06	199.00 b ±3.03	33.77 b ±1.09	42.28 b ±2.29
	Nys.+Propolis Gp.(6)	5.86 b ±0.18	4.02 ab ±0.07	1.84 b ±0.17	186.04 c ±4.29	28.60 b ±1.64	35.43 cd ±2.14
	F-test	*	*	*	**	*	**
	LSD	0.41	0.34	0.29	11.29	6.35	6.53
2nd experimental period	Control Gp.(1)	5.84 b ±0.11	3.95 ab ±0.15	1.89 b ±0.06	198.40 b ±8.45	32.00 bc ±2.00	41.78 c ±1.09
	Itraconazole Gp.(2)	2.64 c ±0.15	1.64 c ±0.11	1.00 c ±0.07	360.40 a ±15.04	66.00 a ±4.00	81.77 a ±3.32
	Nystatin Gp.(3)	5.56 b ±0.09	3.69 b ±0.13	1.87 b ±0.07	192.60 b ±3.09	32.00 bc ±2.00	37.33 cd ±3.01
	Propolis Gp.(4)	6.91 a ±0.06	4.22 a ±0.12	2.69 a ±0.07	144.80 c ±20.46	26.00 c ±2.45	33.77 d ±1.09
	Itra.+Propolis Gp.(5)	5.71 b ±0.09	3.91 ab ±0.15	1.81 b ±0.10	213.40 b ±9.48	36.00 b ±2.45	50.66 b ±2.27
	Nys.+Propolis Gp.(6)	5.93 b ±0.09	4.09 a ±0.11	1.84 b ±0.06	149.40 c ±11.13	26.00 c ±2.45	35.33 cd ±2.44
	F-test	**	**	*	**	**	**
	LSD	0.30	0.38	0.21	36.52	7.72	6.91
3rd experimental period	Control Gp.(1)	5.64 ab ±0.16	4.12 a ±0.07	1.52 b ±0.09	212.00 b ±9.57	35.57 b ±2.81	41.77 b ±1.78
	Itraconazole Gp.(2)	4.92 c ±0.05	3.71 b ±0.07	1.21 c ±0.02	265.40 a ±11.78	50.66 a ±1.78	50.66 a ±2.27
	Nystatin Gp.(3)	5.38 b ±0.10	3.82 a ±0.03	1.56 ab ±0.07	197.40 b ±5.35	40.89 b ±1.66	38.22 b ±1.09
	Propolis Gp.(4)	5.76 a ±0.10	4.07 a ±0.08	1.69 a ±0.04	202.20 b ±10.56	38.22 b ±4.35	39.11 b ±1.66
	Itra.+Propolis Gp.(5)	5.50 ab ±0.07	3.84 a ±0.09	1.66 ab ±0.02	211.40 b ±12.90	42.66 ab ±3.33	40.00 b ±1.99
	Nys.+Propolis Gp.(6)	5.47 ab ±0.06	3.86 a ±0.06	1.62 ab ±0.01	209.80 b ±9.86	39.10 b ±1.99	39.11 b ±2.18
	F-test	*	*	*	*	*	*
	LSD	0.31	0.39	0.15	30.00	8.24	5.45

Means in the same column and in same period followed by different letters are statistically significant and the highest value is represented with the letter a.

LSD: Least significant difference. *: Significant at 0.05 probability. **: Highly significant at 0.01 probability

Table 4. Some kidney function tests (mean values ± SE) in gps.1-6 at the end of 1st ,2nd and 3rd experimental periods .

Periods	Parameters / Gps.	Urea mg/dl	Creatinine mg/dl	Serum electrolytes				
				Ca(mg/dl)	Ph(mg/dl)	Mg(mg/dl)	Na(mEq/l)	K(mEq/l)
1st experimental period	Control Gp.(1)	36.50 bc ±0.96	1.48 b ±0.14	10.80 b ±0.13	4.46 b ±0.18	2.10 ±0.17	138.20 a ±2.08	5.19 bc ±0.08
	Itraconazole Gp.(2)	42.50 a ±0.46	2.52 a ±0.08	11.44 a ±0.16	5.51 a ±0.10	2.22 ±0.19	129.80 c ±0.86	6.02 a ±0.12
	Nystatin Gp.(3)	37.50 bc ±0.26	1.32 b ±0.05	10.40 b ±0.13	4.36 b ±0.14	2.04 ±0.13	138.60 a ±1.60	4.84 c ±0.20
	Propolis Gp.(4)	35.50 c ±0.97	1.38 b ±0.10	10.48 b ±0.34	4.14 b ±0.04	2.22 ±0.17	136.20 ab ±1.28	5.14 bc ±0.09
	Itra.+Propolis Gp.(5)	38.66 b ±0.62	1.56 b ±0.07	10.72 b ±0.15	5.21 a ±0.12	2.18 ±0.12	133.00 bc ±1.00	5.54 ab ±0.14
	Nys.+Propolis Gp.(6)	35.66 c ±0.49	1.31 b ±0.06	10.42 b ±0.15	4.19 b ±0.06	1.98 ±0.11	136.20 ab ±1.24	4.70 c ±0.31
	F-test	*	*	*	*	NS	*	*
	LSD	2.99	0.26	0.56	0.34	-	4.10	0.51
2nd experimental period	Control Gp.(1)	36.22 bc ±0.44	1.41 b ±0.05	11.60 c ±0.29	4.48 c ±0.24	2.04 ±0.13	137.40 ab ±3.57	5.17 bc ±0.16
	Itraconazole Gp.(2)	45.88 a ±1.36	2.85 a ±0.16	14.20 a ±0.34	6.04 a ±0.20	2.45 ±0.17	118.40 d ±2.50	6.28 a ±0.33
	Nystatin Gp.(3)	37.10 b ±0.75	1.63 b ±0.03	11.00 c ±0.29	4.60 bc ±0.12	1.70 ±0.13	140.00 a ±2.85	4.94 bc ±0.26
	Propolis Gp.(4)	34.44 c ±0.75	1.60 b ±0.07	11.50 c ±0.42	4.22 c ±0.09	2.17 ±0.17	134.60 ab ±1.50	4.70 c ±0.31
	Itra.+Propolis Gp.(5)	36.66 bc ±0.61	1.64 b ±0.09	12.80 b ±0.26	5.04 b ±0.15	2.09 ±0.21	126.80 c ±2.71	5.32 b ±0.16
	Nys.+Propolis Gp.(6)	34.66 c ±0.65	1.60 b ±0.04	11.90 bc ±0.29	4.27 c ±0.11	2.18 ±0.07	130.60 bc ±1.50	4.90 bc ±0.17
	F-test	*	**	*	*	NS	*	*
	LSD	2.38	0.25	0.93	0.48	-	7.44	0.47
3rd experimental period	Control Gp.(1)	34.47 bc ±0.77	1.12 ab ±0.15	10.00 ±0.79	4.47 bc ±0.16	2.12 ±0.11	142.20 a ±2.52	5.14 ab ±0.14
	Itraconazole Gp.(2)	39.99 a ±0.89	1.44 a ±0.13	12.00 ±0.94	5.48 a ±0.15	2.32 ±0.10	122.00 b ±2.21	5.42 a ±0.12
	Nystatin Gp.(3)	37.10 ab ±1.05	0.92 b ±0.08	10.00 ±0.61	4.38 bc ±0.09	1.99 ±0.19	142.00 a ±1.41	4.78 b ±0.14
	Propolis Gp.(4)	33.15 c ±1.47	1.08 b ±0.15	11.50 ±1.27	4.11 c ±0.05	2.04 ±0.08	141.00 a ±2.00	4.74 b ±0.17
	Itra.+Propolis Gp.(5)	35.26 bc ±0.87	0.80 b ±0.13	10.00 ±0.61	4.72 b ±0.21	2.05 ±0.20	142.40 a ±0.93	5.40 a ±0.13
	Nys.+Propolis Gp.(6)	35.52 bc ±1.25	0.88 b ±0.05	10.50 ±0.94	4.09 c ±0.10	2.07 ±0.21	143.40 a ±1.08	5.14 ab ±0.13
	F-test	*	*	NS	*	NS	*	*
	LSD	3.14	0.35	-	0.40	-	5.23	0.41

Means in the same column and in same period followed by different letters are statistically significant and the highest value is represented with the letter a. LSD: Least significant difference. NS:Non significant changes. *: Significant at 0.05 probability.

**: Highly significant at 0.01 probability

Table 5. Erythrogram and leukogram (mean values ± SE) in gps. 1-6 at the end of the 1st, 2nd and 3rd experimental periods .

Periods	Parameters / Gps.	RBCs (10^6/µl)	Hb (gm%)	PCV (%)	MCV (fl)	MCHC (%)	TLC (10^3/µl)	Differential leukocytic count × 10^3 µl			
								Neut	Lymph.	Eosin.	Mono.
1st experimental period	Control Gp.(1)	7.00 ab ±0.23	13.32 b ±0.10	34.40 b ±0.24	49.35 b ±1.63	38.72 a ±0.22	8.68 ±0.34	3.40 ±0.08	4.90 ab ±0.36	0.09 c ±0.03	0.29 ±0.02
	Itraconazole Gp.(2)	5.13 c ±0.31	11.00 c ±0.45	31.80 c ±0.58	63.15 a ±4.97	34.61 b ±1.41	8.28 ±0.39	3.53 ±0.15	4.06 b ±0.28	0.36 a ±0.007	0.24 ±0.01
	Nystatin Gp.(3)	6.30 b ±0.26	13.56 b ±0.32	34.00 b ±0.45	–	–	7.75 ±0.54	2.88 ±0.31	4.44 b ±0.25	0.09 c ±0.01	0.26 ±0.02
	Propolis Gp.(4)	7.39 a ±0.29	16.44 a ±0.57	38.80 a ±0.97	–	–	9.24 ±0.40	3.28 ±0.27	5.46 a ±0.26	0.18 b ±0.04	0.33 ±0.01
	Itra.+Propolis Gp.(5)	5.09 c ±0.21	11.40 c ±0.30	32.00 c ±0.68	62.87 a ±1.64	35.63 b ±0.48	9.04 ±0.35	3.07 ±0.21	5.47 a ±0.24	0.17 b ±0.01	0.29 ±0.02
	Nys.+Propolis Gp.(6)	6.31 b ±0.26	14.48 b ±0.43	35.40 b ±0.68	–	–	8.32 ±0.16	3.64 ±0.11	4.34 b ±0.13	0.09 c ±0.01	0.25 ±0.02
	F-test	*	*	*	*	*	NS	NS	*	**	NS
	LSD	0.77	1.14	1.87	7.08	2.09	-	-	1.00	0.06	-
2nd experimental period	Control Gp.(1)	6.54 b ±0.27	13.24 b ±0.34	35.00 c ±0.32	53.78 b ±1.65	37.80 a ±0.66	9.07 bc ±0.32	3.30 b ±0.15	5.30 ab ±0.36	0.09 c ±0.004	0.29 ±0.02
	Itraconazole Gp.(2)	3.45 c ±0.17	10.20 c ±0.50	32.80 d ±0.37	92.75 a ±4.21	31.10 b ±1.09	7.68 c ±0.62	2.17 c ±0.15	4.58 b ±0.59	0.64 a ±0.02	0.23 ±0.02
	Nystatin Gp.(3)	6.03 b ±0.17	13.20 b ±0.14	34.00 c ±0.55	–	–	8.62 bc ±0.32	3.79 b ±0.38	4.45 b ±0.14	0.11 c ±0.006	0.25 ±0.008
	Propolis Gp.(4)	7.49 a ±0.20	17.72 a ±0.22	40.80 a ±0.97	–	–	11.82 a ±0.75	5.19 a ±0.49	6.16 a ±0.26	0.07 c ±0.007	0.37 ±0.02
	Itra.+Propolis Gp.(5)	6.43 b ±0.28	13.20 b ±0.32	34.20 c ±0.37	–	–	9.80 b ±0.70	3.56 b ±0.30	5.50 ab ±0.40	0.32 b ±0.02	0.36 ±0.01
	Nys.+Propolis Gp.(6)	7.11 ab ±0.29	16.76 a ±0.54	38.60 b ±0.87	–	–	8.53 bc ±0.30	3.51 b ±0.09	4.57 b ±0.29	0.09 c ±0.006	0.34 ±0.01
	F-test	*	*	*	**	*	*	*	*	**	NS
	LSD	0.69	1.08	1.84	6.06	1.90	1.57	0.87	1.07	0.05	-
3rd experimental period	Control Gp.(1)	7.26 a ±0.14	14.16 a ±0.28	36.60 a ±0.24	50.46 b ±0.68	38.67 a ±0.52	9.00 ab ±0.28	4.00 ab ±0.14	4.60 ab ±0.15	0.10 c ±0.006	0.30 ±0.007
	Itraconazole Gp.(2)	3.85 b ±0.41	10.84 b ±0.32	30.00 b ±0.63	77.92 a ±7.79	36.13 b ±0.41	7.96 b ±0.49	2.78 c ±0.47	4.45 ab ±0.19	0.47 a ±0.02	0.26 ±0.01
	Nystatin Gp.(3)	7.17 a ±0.20	13.72 a ±0.27	36.00 a ±0.45	–	–	8.26 b ±0.10	3.50 b ±0.08	4.41 ab ±0.14	0.06 c ±0.005	0.32 ±0.004
	Propolis Gp.(4)	6.61 a ±0.26	13.76 a ±0.17	35.60 a ±0.51	–	–	10.35 a ±0.13	4.65 a ±0.18	5.40 a ±0.09	0.06 c ±0.02	0.30 ±0.02
	Itra.+Propolis Gp.(5)	6.60 a ±0.21	13.45 a ±0.19	35.40 a ±0.51	–	–	8.47 b ±0.25	3.74 b ±0.17	4.21 b ±0.14	0.22 b ±0.02	0.30 ±0.007
	Nys.+Propolis Gp.(6)	7.00 a ±0.09	13.64 a ±0.29	35.60 a ±0.24	–	–	8.82 b ±0.31	3.56 b ±0.18	4.87 ab ±0.21	0.09 c ±0.006	0.31 ±0.01
	F-test	*	*	*	*	*	*	**	*	**	N S
	LSD	0.70	0.76	1.33	9.85	1.44	1.50	0.70	1.00	0.04	-

Means in the same column and in same period followed by different letters are statistically significant and the highest value is represented with the letter a. LSD: Least significant difference. NS:Non significant changes. *: Significant at 0.05 probability.

**: Highly significant at 0.01 probability

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الملخص العربي

دراسات باثولوجية إكلينيكية على تأثير الأدوية المضادة للفطريات في الأرانب

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أجريت هذه الدراسة على بعض مضادات الفطريات المصنعة (اتراكونازول والنيستاتين)و الطبيعية(البروبليز) وخليط من (الأتراكونازول والبروبليز)وكذلك (النيستاتين و البروبليز) لمعرفة التأثيرات الجانبية المختلفة لكل عقار على حده و بعد استخدامه مع البروبليز.وقد أجريت هذه الدراسة على عدد ٦٠ أرنب وقد قسمت هذه الأرانب الى ست مجموعات كل مجموعة تحتوي على عدد ١٠ أرانب وقد تركت المجموعة الأولى كمجموعة ضابطة للتجربة أما المجموعة الثانية فقد تم تجريعها بالاتراكونازول والثالثة فقد تم تجريعها بالنيستاتين والرابعة بالبروبليز أما المجموعة الخامسة فقد تم تجريعها بخليط من انصاف الجرعات الدوائية لكل من الأتراكونازول والبروبليزأما المجموعة السادسة فقد تم تجريعها بخليط من انصاف الجرعات الدوائية لكل من النيستاتين والبروبليز وكان ذلك يوميا لمدة شهر وقد تم أخذ العينا ت عند اليوم ١٥ و٣٠من تعاطي الأدوية وعند اليوم ٧ بعد توقف تعاطي الأدوية.

وقد وجد أن الأتراكونازول سبب أضرارا كبدية حيث أدى الى زيادة في أنزيمات الألانين أمينو ترانسفيريز والاسبرتيت أمينو ترانسفيريز والفوسفاتيز القلوي و البيليروبين الكلي والمباشر وغير المباشر ونقص معنوي في قيمة البروتينات الكلية والألبيومين و الجلوبيولين طوال مدة التجربة.كما أدى الى زيادة معنوية في الدهون الكلية والكوليستيرول والدهون الثلاثية طوال فترات التجربة. كما سبب أضرارا كلوية تمثلت في زيادة معنوية في قيمة اليوريا والكرياتينين بالسيرم في المجموعة الثانية طوال فترات التجربة وبمشاهدة مستويات الألكترولايت في السيرم لوحظ زيادة معنوية في قيم الكالسيوم والفسفوروالبوتاسيوم ونقص في مستوى الصوديوم .بالأضافة الى أنيميا ونقص في الخلايا متعادلة الصبغة مرتبطة بزيادة في الخلايا حامضية الصبغة.أما النيستاتين فلم يسبب أضرارا جانبية . وقد وجد أن البروبليز حسن من وظائف الكبد والكلى وصورة الدم .وعند استخدامه مع الأتراكونازول بأنصاف الجرعات الدوائية قلل من التغيرات الكيميائية والهيماتولوجية . وقد وجد أن التغيرات في صورة الدم والسيرم الناتجة عن استخدام الأدوية المضادة للفطريات تحتاج الى أكثر من سبعة أيام حتى تعود الى الحالة الطبيعية.

لذلك يوصى بأستخدام الأتراكونازول مع البروبليز بأنصاف الجرعات الدوائية للتقليل من الآثار الجانبية للأتراكونازول .أيضا استخدام النيستاتين مع البروبليز بأنصاف الجرعات الدوائية يوسع من دائرة استخدام النيستاتين.