Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 11(2), 913-933, 2003

EFFECT OF DINICONAZOLE ON METABOLIC PROFILE OF ALBINO RATS

[65]

Zidan[1], Z.H.; M.M. El-Herawie[2]; S.I. Ghoneim[3] and M.M. El-Said[2]

ABSTRACT

This study was conducted to evaluate the subchronic toxicity of diniconazole fungicide in male albino rats. Diniconazole caused a significant decrease in the activity of aspartate aminotransferase (AST) on the 90th day of treatment, while, the alanine aminotransferase (ALT) did not alter significantly throughout the experiment. Plasma alkaline phosphatase (ALP) activity showed a marked elevation after treatment with diniconazole, after the recovery period the values returned to the normal levels. The creatine phosphokinase (CPK) decreased significantly after 15 days, but increased after 30 days from treatment and approached to normal values during the recovery period. Diniconazole treatments did not alter significantly urea, uric acid, total protein, albumin and magnesium levels while creantinine and glucose levels declined in treated rats after 90 and 45 days, respectively. In addition, plasma total lipids, total cholesterol and triglycerides levels were varied throughout the experiment. Treatments with diniconazole for 90 days produced a slight toxic effect on parathormone level and calcium profile as well as the inorganic phosphorus level. Diniconazole induced a significant increase of body and brain weights while, the weights of liver, lung, heart, kidney, spleen and testes did not change throughout the experiment. A moderate damage in kidney tissues in diniconazole treated rats was also observed.

Key words: Rats, Diniconazole, Fungicide, Metabolism, Enzymes

INTRODUCTION

In developing countries extensive use of pesticides to meet with increased agricultural needs is inevitable and the indiscriminate use has led to several toxicological implications in humans.

The main hazards of fungicides on human and domestic animals is likely to rise from their use as seed dressing for the protection of stored crops, and for example the diniconazole is used as fungicide in crop protection because of its systemic activity against the fungal plant disease. The information describing the possible deleterious effects of diniconazole is scarce particularly in mammals. For this, the present work was conducted

1- Fac. of Agric., Ain Shams University, Shoubra El-Kheima, Cairo, Egypt
2- Central Laboratory of Pesticides, (MOA), Dokki, Giza, Egypt.
3- Fac. of Agric. Suez Canal University, Ismalia, Egypt.

(Received July 19, 2000)
(Accepted May 26, 2003)

to evaluate diniconazole fungicide effect on constituents in metabolic profile of male albino rats.

MATERIAL AND METHODS

1- Experimental animals

The present study was performed on 120 adult male albino rats of average weight (80-100 g). Animals were obtained from General Organization of Serum Products and Vaccine (Helwan Farm), Cairo, Egypt. These animals were clinically healthy and housed in hygenic conditions and fed on balanced diet, **(El-Maassarawy, 1996)**. Rats were acclimatized under the test conditions for two weeks before fungicide treatment.

2- Acute toxicity experiment

The procedure for median lethal dose (LD_{50}) of diniconazole of **Weill, (1952)** was followed in the present investigation.

3- Subchronic toxicity experiment

The animals were allocated into four main groups. The first was considered as a control group and the other three groups represented the concentrations tested, i.e., one group per concentration. The tested compound diniconazole fungicide [E-1-2,4 dichlorophenyl) 4-4-dimethyl-2-(1,2,4-triazole-1-yl] 1-penten-3-ol] was dissolved in drinking water and provided for the animals *ad libitum;* the active ingredient in the water was adjusted to 12, 25 and 50 ppm. Each of the main group was divided randomly into six subgroups representing the treatment periods; 15, 30, 45, 60 and 90 days. The fungicide was withdrawn for 30 days to allow recovery from toxicity. The body weight of the animals was recorded every week. At the end of each period, the blood samples were collected from sacrafficial rats, in small labelled dry tubes containing heparin (10 IU/ml fresh blood) **[Schalm, 1986]**. The weights of some internal organs were recorded in fresh condition. The sample of kidney was collected and immersed in Smith solution as fixative for anatomopathological examinations. Blood was centrifuged at 3500 rpm for 15 minutes, in refrigerated centrifuge to separate plasma which was divided into aliquots and kept under -20°C.

4- Biochemical determinations

4.1. The activities of aminotransferases (ALT & AST) and alkaline phosphatase were determined colorimetrically **(Reitman and Frankel 1957; Hausdman, *et al* 1967)**, respectively, using kits supplied by Bio-Muriex (France).

4.2. Plasma urea, uric acid and creatinine concentrations were determined according to the methods of **Coulombe and Farreau (1963); Triwedi *et al* (1978) and Husdan and Rapoport (1968)**, respectively.

4.3. Total plasma protein and albumin concentrations were determined according to the method of **Weichselbaum (1946) and Drupt (1974)**, respectively.

4.4. Plasma total cholesterol, triglycerides and total lipids concentrations were determined according to the methods of **Charles *et al* (1974), Fossati and Prencipe (1988)** and **Frings and Dum (1970)**, while the glucose concentration was estimated according to the method of **Darham and Trinder (1972).**

4.5. Plasma creatine phosphokinase activity was determined according to the method of **Morin (1977).**

4.6. As for calcium profile and related regulators, the total calcium concentration was determined according to the method of **Robertson and Marshall (1979),** while the free ionized calcium concentration was calculated indirectly by using Zeisler's formula **[Puerro and Alexandre, 1995]**. The bound calcium was calculated by subtracting the value of plasma free ionized calcium from the total calcium concentration.

4.7. Determination of plasma inorganic phosphorus concentration and plasma magnesium concentration was determined according to the method of **Bohuon (1962).**

4.8. The parathormone hormone was assayed by using Radio immunoassay kits (RIA) according to the method of **Hawker and Dibella (1980).**

5. Statistical analysis

All data were subjected to statistical analysis according to the procedure reported by the **Snedecor and Cochran (1980).** Also, statistical analysis of some data was carried out according to the method of **Dixon and Massay (1957).**

RESULTS

1- Biochemical changes in diniconazole treated rats

Oral administration of diniconazole in rats produced a significant decline in the activity of aspartate aminotransferase (AST) at 12 and 25 ppm 90 days after treatment. During the recovery period (30 days), the values of AST returned to the control values. Alanine aminotransferase (ALT) did not alter significantly throughout the experiment. (Table 1).

Data in Table (1) show that the activity of alkaline phosphatase (ALP) was significantly elevated when diniconazole was administered to rats at 12 and 25 ppm for 30 days. The same trend was observed at 12 ppm after 90 days from treatment. Again the values of this enzyme returned to the normal level during the recovery period. The diniconazole treatments resulted in a significant decrease in the activity of creatine phosphokinase (CPK) after 15 days, while the activity of CPK increased at 25 and 50 ppm of diniconazole after 30 days of treatment. During the recovery period the activity of CPK approached normal level. Data in Table (2) indicate that diniconazole did not cause any significant differences in urea and uric acid concentrations. On the other hand, the creatinine level was markedly declined in rats treated with diniconazole (at 25 ppm) after 90 days.

Data in Table (3) indicate that diniconazole did not cause any adverse effects on the total protein and albumin concentrations in rats. The results show that there was significant decrease in plasma glucose level in treated rats. The same pattern was observed within the first 2 weeks from treatment but the animals recovered to normal level. Data in Table (4) show that the total lipids content was significantly increased after 30 and 45 days in rats treated with 12 and 25 ppm. All treated animals recovered and lipids content reached normal level. Also, diniconazole treatments induced marked increase in triglycerides content after 15 days (12 and 25 ppm) and 60 days (12 and 50 ppm). On the other hand, there

Table 1. Effect of treatment with different concentrations of diniconazole on liver function Creatinine phosphokinase activity of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Aspartate aminotransferase activity (u/L)							
Control (0.0 ppm)	160.600	160.000	160.000	147.400	154.600	169.620	158.753
12 ppm	161.100	167.600	162.700	156.000	143.20*	159.200	156.633
25 ppm	169.160	158.000	168.300	141.700	145.76*	162.800	157.620
50 ppm	161.800	166.700	164.800	144.600	155.000	162.600	159.250
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 10.96							
Alanine aminotransferase activity (u/L)							
Control (0.0 ppm)	62.980	42.900	59.634	32.100	49.374	46.374	48.891
12 ppm	63.300	60.680	57.100	33.400	34.400	38.700	47.930
25 ppm	60.766	52.200	56.200	27.200	32.800	42.100	45.211
50 ppm	63.800	43.200	50.500	33.700	51.458	44.600	48.894
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							
Alkaline phosphatase activity u/L)							
Control (0.0 ppm)	127.868	90.000	144.28	103.424	117.830	121.706	117.643
12 ppm	130.856	129.152*	121.182	120.442	135.84*	82.314	119.965
25 ppm	111.30	128.472*	152.032	112.110	103.064	100.154	118.494
50 ppm	135.34	87.870	125.364	131.204	131.240	125.684	122.749
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 31.189							
Creatinine phosphokinase activity (u/L)							
Control (0.0 ppm)	3946.77	863.442	316.72	142.104	129.156	952.355	952.355
12 ppm	2194.0*	501.236	655.696	194.220	116.554	153.692	669.23*
25 ppm	2936.60	1745.6*	860.656	114.620	361.312	241.398	1041.70
50 ppm	1364.81	2660.6*	671.936	178.752	193.920	157.168	787.874
L.S.D. of treatment means = 251.6103. L.S.D. of interaction between period of treatment x treatment concentration - 616.317.							

Table 2. Effect of treatment with different concentrations of diniconazole on kidney function of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Urea concentration (mg/dL)							
Control (0.0 ppm)	25.764	31.812	35.756	42.378	37.200	34.890	34.800
12 ppm	37.326	34.580	37.742	42.950	41.162	34.530	38.048
25 ppm	33.040	37.746	37.532	45.726	36.346	34.330	37.453
50 ppm	37.764	31.812	35.756	42.378	37.200	35.890	34.800
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							
Uric acid (mg/dL)							
Control (0.0 ppm)	13.970	12.494	12.326	16.522	13.866	13.652	13.802
12 ppm	13.508	16.304	15.838	20.492	15.582	15.022	10.340
25 ppm	10.660	11.220	16.802	24.770	15.050	13.838	15.387
50 ppm	10.250	15.746	16.064	19.416	15.616	12.204	14.883
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							
Creatinine concentration (mg/dL)							
Control (0.0 ppm)	8.672	5.724	6.488	10.766	12.032	11.760	9.574
12 ppm	7.122	7.992	5.548	9.250	12.840	11.826	9.096
25 ppm	7.798	4.512	6.246	8.500	11.400	8.950	7.901*
50 ppm	7.202	4.920	5.682	11.244	12.300	10.760	8.620
L.S.D. of treatment means = 0.9794 No significant of interaction between period of treatment x treatment concentration							

Table 3. Effect of treatment with different concentrations of diniconazole on total protein, albumin conc. and glucose conc. of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Total protein conc. (g/dL)							
Control (0.0 ppm)	6.334	5.484	5.458	5.326	5.538	5.714	5.914
12 ppm	6.216	6.164	5.058	5.246	5.260	4.864	5.528
25 ppm	6.618	5.460	5.454	5.192	6.058	5.088	5.210
50 ppm	6.664	4.544	5.498	6.128	6.366	5.276	5.390
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							
Albumin conc. (g/dL)							
Control (0.0 ppm)	2.640	3.694	2.030	2.736	3.260	2.850	2.702
12 ppm	3.154	3.030	3.042	2.870	2.506	2.324	2.821
25 ppm	3032	2.170*	3.048	2.362	2.560	2.274	2.831
50 ppm	3.172	1.680	2.860	2.954	2.506	2.544	3.619
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 0.723							
Glucose conc. (mg/dL)							
Control (0.0 ppm)	76.970	68.162	108.352	155.308	130.176	135.428	112.399
12 ppm	52.222*	63.276	46.28*	118.34*	117.532	127.240	87.483
25 ppm	104.25*	72.110	74.58*	146.268	149.630	138.312	114.195
50 ppm	72.456	68.130	85.37*	145.354	141.456	128.164	106.822
L.S.D. of treatment means = 8.4580 L.S.D. of interaction between period of treatment x treatment concentration = 20.718							

Table 4. Effect of treatment with different concentrations of diniconazole on lipid profile of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Total lipid conc. (g/dL)							
Control (0.0 ppm)	1.864	1.284	1.474	2.426	2.104	2.746	1.983
12 ppm	1562	2.474*	1.384*	1.980	2.032	2.512	1.991
25 ppm	1.606	1.956	2.384	1.846	2.570	2.794	2.195
50 ppm	2.402	1.900	1.908	2.322	1.944	2.802	2.213
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 0.684							
Triglycerides conc. (mg/dL)							
Control (0.0 ppm)	42.668	75.604	78.852	55.898	82.976	78.024	69.004
12 ppm	49.808*	80.776	87.158	76.028*	63.484*	75.154	72.068
25 ppm	62.380	68.864	81.232	69.584	68.206	93.404	73.945
50 ppm	89.114*	83.406	70.444	85.746*	79.042	75.476	80.538*
L.S.D. of treatment means = 7.562 L.S.D. of interaction between period of treatment x treatment concentration = 18.525							
Cholesterol level (mg/dL)							
Control (0.0 ppm)	40.149	51.118	53.200	49.166	47.962	52.576	49.027
12 ppm	42.902	64.318*	41.364*	40.068	39.400*	52.730	46.797
25 ppm	31.096	41.482	49.744	40.550	39.062*	59.282	43.536
50 ppm	34.410	40.26	51.686	49.132	51.600	50.106	46.200
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 10.975							

was a significant decrease in triglycerides after 90 days of treatment at 12 and 25 ppm of diniconazole. After the recovery period treated animals returned to normal levels.

Table (4), shows that diniconazole caused significant differences in total of treated rats with the lower and medium concentrations (12 and 25 ppm) but animals recovered by the end of the experiment. Data indicate that diniconazole treatments, in general, did not alter parathormone (PTH) level throughout the experimental periods. The lowest concentration of this fungicide showed significant reduction in PTH level after 60 days from treatment, while the medium concentration caused significant elevation in PTH level after 90 days of treatment These animals became normal. The highest concentration did not cause any change in PTH level, (Table, 5).

Total calcium concentration significantly decreased after 15 days of treatment with 12 and 25 ppm of diniconazole. Whereas, there was an increase in calcium concentration after 60 days with the highest concentration (50 ppm); and complete recovery. The same trend of results occurred with free-ionized calcium (Ca^{2+}) during the experimental period. (Table, 5). All concentrations of diniconazole did not induce any significant differences in bound calcium.

Data in Table (5) indicate that diniconazole treatments resulted in significant decrease in inorganic phosphate at concentrations of 12 and 50 ppm and after withdrawal of diniconazole the values returned to normal levels. Results show that the plasma magnesium (Mg^{2+}) level did not differ significantly than the control group.

2. Body and organs weights changes

Data in Table (6) indicate that there was difference, in general, in body weight gain between diniconazole treated rats and untreated animals. The highest concentration of diniconazole (50 ppm) caused significant reduction in body weight of treated rats than control after 60, 70 and 90 days of treatment. Recovery occurred within the recovery period.

Data in Tables (7; 8) indicate that the higher concentrations of the diniconazole (25 and 50 ppm) caused significant increase in weights of brain. Whereas, the diniconazole treatments did not produce any significant changes in weights of lung, liver, heart, kidney, spleen and testes throughout the experiment.

3. Histopathological changes in kidney

A moderate changes were observed in the kidney of the treated rats with diniconazole (Figs, 1-5).

DISCUSSION

Damage to variety of animal tissues resulted in increased blood levels of AST, ALT and ALP enzymes but these were not always changes specific, in the sense that several organs or tissues contained similar enzymes but did not implicate a particular organ.

Enzymes of diagnostic value circulating in the blood have no physiological function. Even in normal animals in the absence of pathological lesions, the continual processes of cell replication, growth and decay give a base line of blood enzymes concentration. Enzymes in the blood fulfil no metabolic role, but rather indicate indirectly the metabolic function.

Table 5. Effect of treatment with different concentrations of diniconazole on calcium profile and calcium related regulators in male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Over all mean of treatment
	Parathormone (PTH) (mg/dL)						
Control (0.0 ppm)	8.162	8.010	7.274	7.608	7.890	11.194	8.360
12 ppm	6.202	6.140	7.942	8.402	7.780	8.934	7.567
25 ppm	6.222	6.264	7.206	6.420	8.268	7.326	6.951
50 ppm	7.190	6.646	7.588	9.630	8.874	8.368	8.049
L.S.D. of treatment means = 0.786. L.S.D. of interaction between period of treatment x treatment concentration = 1.927.							
	Calcium concentration (mg/dL)						
Control (0.0 ppm)	4.184	4.280	3.650	3.588	3.856	5.612	4.200
12 ppm	2.880	3.100	3.678	4.322	4.080	5.072	3.856
25 ppm	2.622	3.560	3.786	3.168	4.358	3.780	3.547
50 ppm	3.538	3.208	3.644	4.992	4.460	4.404	4.041
L.S.D. of treatment means = 0.4724. L.S.D. of interaction between period of treatment x treatment concentration = 1.157.							
	Free ionized calcium (mg/dL)						
Control (0.0 ppm)	3.970	3.750	3.624	4.020	4.034	5.552	4.158
12 ppm	3.322	2.852	4.264	4.100	3.700	3.862	3.683
25 ppm	3.660	2.776	3.450	3.252	3.910	3.540	3.424
50 ppm	3.652	3.428	3.944	4.618	4.414	3.964	4.005
L.S.D. of treatment means = 0.4486. No wignificant of interaction between period of treatment x treatment concentration.							
	Bound calcium (mg/dL)						
Control (0.0 ppm)	4.806	4.884	4.884	5.332	5.240	6.432	5.200
12 ppm	5.226	4.634	4.946	5.098	4.978	6.040	5.319
25 ppm	5.548	5.326	5.130	5.628	4.518	5.698	5.308
50 ppm	5.824	4.196	4.760	5.932	4.762	5.944	5.236
No significant differences exist between treatments. L.S.D. of interaction between period of treatment x treatment concentration - 1.137.							

Table 6. The effect of treatment with different concentrations of diniconazole on body weight gain in male albino rats

Time	Concentration			
	Control	12 ppm	25 ppm	50 ppm
Initial B.W. (0)	75.03±4.25	74.56±2.50	77.80±3.50	68.0±3.65
10 days	83.98±3.89	66.53±3.39	67.23±3.58	62.56±3.45
20 days	80.63±4.33	76.89±2.71	81.58±4.20	81.71±3.46
30 days	104.58±11.17	95.98±7.53	110.26±3.63	101.51±3.91
40 days	144.58±11.17	127.28±11.06	146.41±3.91	121.72±9.49
50 days	160.30±17.39	177.42±4.61	170.75±13.16	157.93±4.45
60 days	211.69±5.67	227.82±6.80	210.56±5.43	167.45±13.52**
70 days	233.57±8.18	245.14±7.80	233.14±8.60	189.42±7.40
80 days	248.44±6.77	256.57±7.25	246.57±7.74	213.42±10.54*
90 days	260.00±8.43	271.00±8.94	251.44±8.50	213.42±10.54
Recovery for 30 day				
100 days	285.60±17.0	290.75±13.43	271.50±9.47	231.25±18.95
110 days	298.10±19.75	277.75±22.59	271.75±23.58	237.75±20.77
120 days	302.25±25.01	303.50±18.66	300.75±19.86	268.00±12.90

Mean ± S.E.
* Mean significantly differ from the control at $P < 0.05$.
** Mean significantly differ from the control at $P < 0.01$.

Table 7: Effect of treatment with different concentrations of diniconazole on brain, lung, heart and liver weights of male albino rats

Treatment	Treatment preiod (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Brain weight (g/100 g b.wt.)							
Control (0.0 ppm)	4.534	4.460	4.432	4.342	4.450	4.274	4.415
12 ppm	4.200	4.804	4.170	4.814	4.438*	3.924	4.375
25 ppm	4.474	4.784	4.526	4.938	4.650*	4.154	4.588*
50 ppm	4.590	4.878	4.540	5.286	4.912*	4.362	4.761*
L.S.D. of treatment means = 0.1389							
L.S.D. of interaction between period of treatment x treatment concentration = 0.340							
Lung weight (g/100 g b.wt.)							
Control (0.0 ppm)	4.828	4.762	5.166	4.516	4.080	4.200	4.592
12 ppm	4.360	4.552	4.242	4.474	4.404	4.266	4.383
25 ppm	4.354	4.292	4.510*	5.214*	4.708	4.276	4.559
50 ppm	4.664	4.382	4.104	4.278	4.362	4.488	4.480
No significant differences exist between treatments							
L.S.D. of interaction between period of treatment x treatment concentration = 0.541							
Heart weight (g/100 g b.wt.)							
Control (0.0 ppm)	3.196	3.652	3.358	3.338	3.046	3.348	3.323
12 ppm	3.446	3.358	3.234	3.626	3.780*	3.144	3.381
25 ppm	3.388	3.466	3.364	3.518	3.606*	3.092	3.406
50 ppm	3.186	3.408	3.164	3.440	3.390	3.366	3.326
No significant differences exist between treatments							
L.S.D. of interaction between period of treatment x treatment concentration = 0.329							
Liver weight (g/100 g b.wt.)							
Control (0.0 ppm)	9.558	11.386	11.442	10.840	10.606	10.438	10.712
12 ppm	10.138*	10.736	10.590*	11.424	10.718	10.236	10.640
25 ppm	10.010	10.860	10.620*	11.238	10.930	10.744	10.734
50 ppm	10.488	10.810	11.390	12.042*	10.868	10.422	11.003
No significant differences exist between treatments							
L.S.D. of interaction between period of treatment x treatment concentration = 0.720							

Table 8. Effect of treatment with different concentrations of diniconazole on kidney, spleen and testes weights of male albino rats

Treatment	Treatment period (days)						
	15	30	45	60	90	Recovery for 30 days	Allover mean of treatment
Kidney weight (g/100 g b.wt.)							
Control (0.0 ppm)	4.410	5.046	5 .426	5.094	4.502	5.078	4.926
12 ppm	4.850	4.612	4.922*	4.726	5.198	5.040	4.891
25 ppm	5.042*	4.816	4.956*	5.410	5.210*	4.762	5.034
50 ppm	4.684	4.628	5.504	5.296	4.558	4.844	4.919
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 0.447							
Spleen weight (g/100 g b.wt.)							
Control (0.0 ppm)	2.568	3.000	3.140	2.576	2.696	2.858	2.761
12 ppm	2.612	2.774	3.582	2.694	2.752	2.696	2.685
25 ppm	2.808	2.934	2.702	2.876	2.962	2.440	2.787
50 ppm	2.908	2.996	2.868	3.026	2.842	2.862	2.905
No significant differences exist between different treatments No significant of interaction between period of treatment x treatment concentration							
Testes weight (g/100 g b.wt.)							
Control (0.0 ppm)	6.614	5.852	5.816	5.772	5.588	5.704	5.891
12 ppm	5.562*	5.908	5.616	6.002	6.118*	5.434	5.773
25 ppm	5.802	5.882	5.876	6.014	6.288*	5.498	5.893
50 ppm	6.216	5.984	5.908	5.876	5.980	5.590	5.926
No significant differences exist between treatments L.S.D. of interaction between period of treatment x treatment concentration = 0.4989							

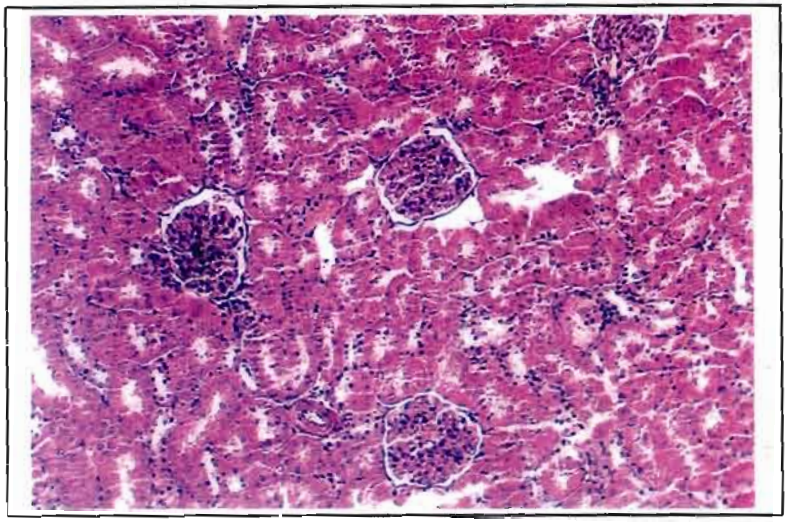

Fig. 1. Cross section in the renal cortex of control rat showing glomeruli and kidney tubules (E & H, 40x).

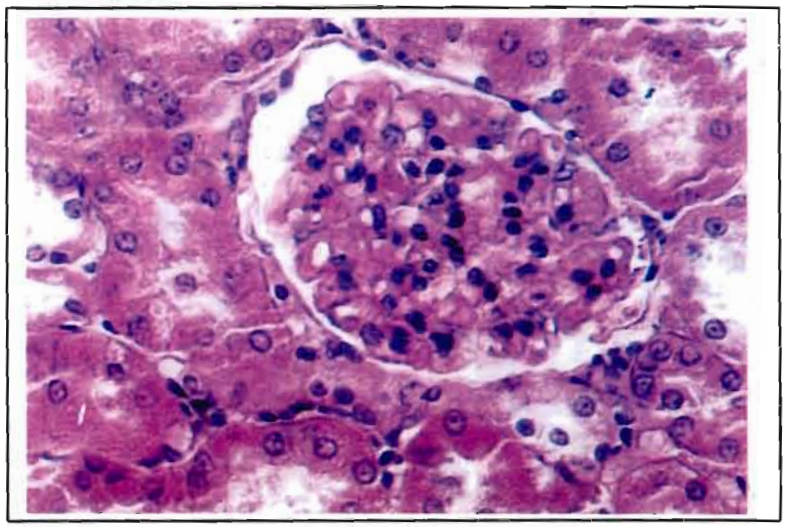

Fig. 2. Cross section in the renal of control rat showing renal glomeruli magnification (E & H 160x).

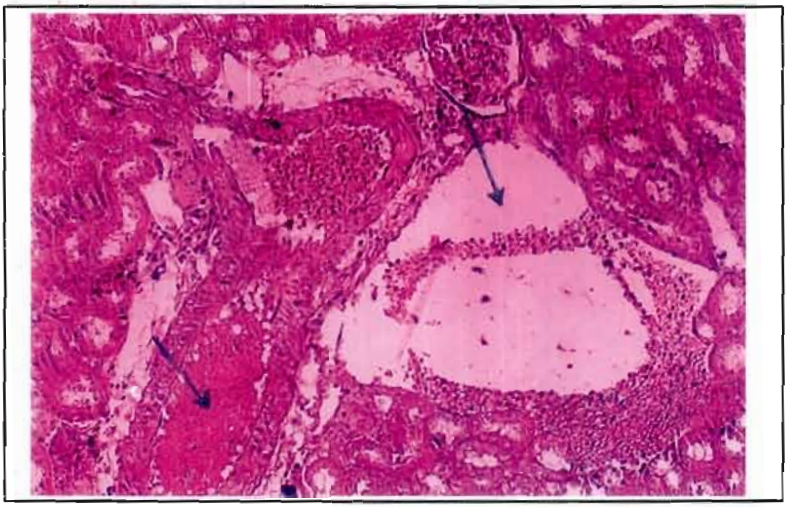

Fig. 3. Cross section in the renal cortex of rat treated with 12 ppm of diniconazole for 15 days showing engorgement of intertubular vessels with blood and rupture of some blood vessels with haemorrhage. (E & H, 40x).

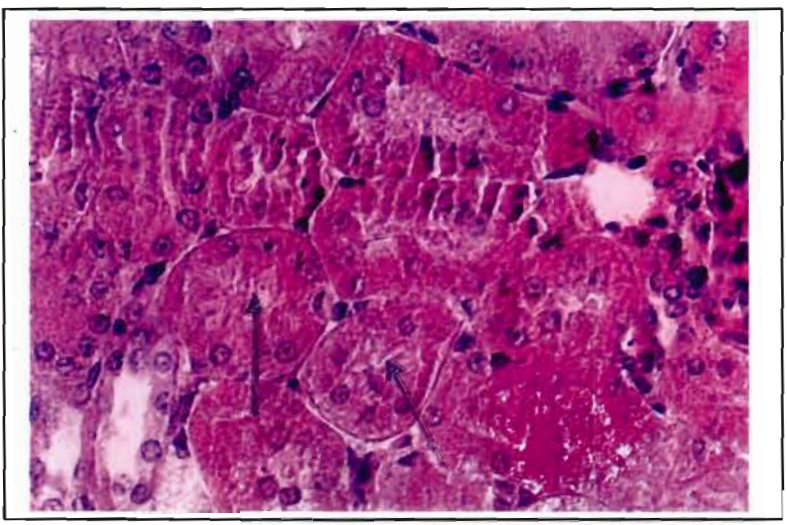

Fig. 4. Cross section in the renal cortex of rat treated with 25 and 50 ppm of diniconazole for 45 days, showing hypertrophy of the epithelial cell lining the kidney tubules with narrowing of the lumen of tubules. (E & H, 40x).

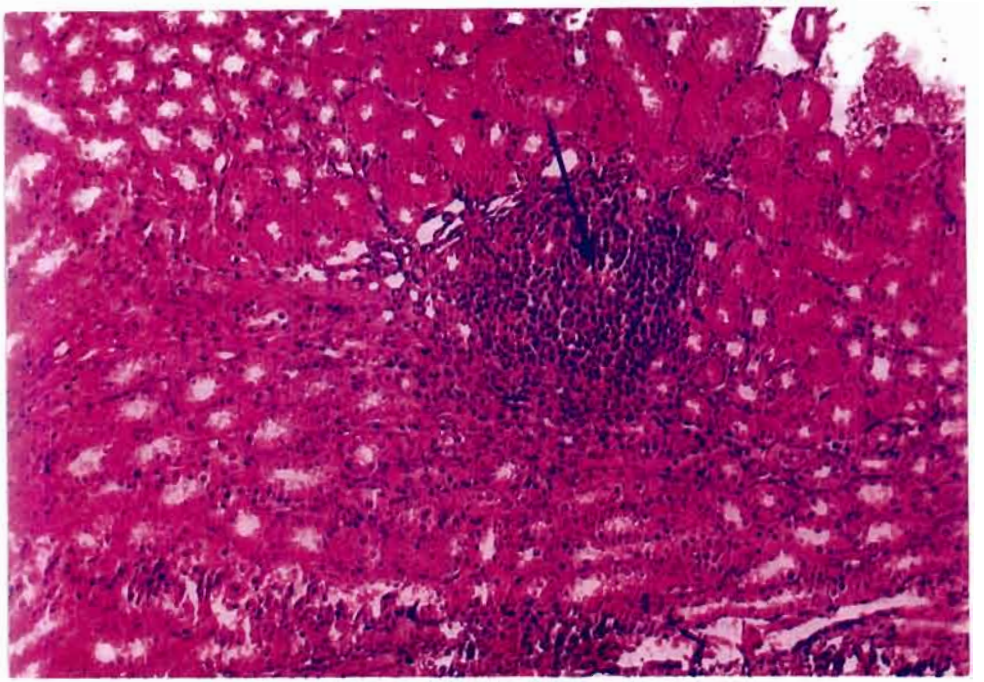

Fig. 5 Cross section in the renal cortex of control rat treated with 12 ppm for 60 days, showing focal leucocytic infiltration. (E & H, 40x).

Results of the present investigation revealed that administration of diniconazole to male rats (90 days), did not alter the plasma activity of analinine aminotransferase (ALT) during the experimental period.

Meanwhile, the plasma activity of aspartate aminotransferase (AST) was significantly reduced on the 90th day of treatment and after withdrawal the diniconazole. This enzyme activity returned to its normal value of the untreated animals. Although, the AST values were statistically lower than the control but it fell within the normal values previously reported by **Kaneko (1989)**.

Moreover, the plasma alkaline phosphatase (ALP) activity was less changed except on the 45th and 90th day after treatment where a marked elevation was noticed; and after withdrawal of pesticide used, the values of this enzyme returned to the normal levels of untreated group.

It is worth mentioning that although the values of ALP were significantly different than the control values but they fell within the normal range of rats **(Kaneko, 1989)**. Results of the present work agree to great extent with those of **Zidan *et al* (1991), Gojmerac *et al* (1995) and Nasr *et al* (1996)** who noted that aspartate aminotransferase (AST) activity was significantly decreased, while no alteration occurred in the activity of alanine aminotransferase (ALT) in rats treated with

several toxicants, i.e. sumicidin, atrazine and phoxim. Moreover, alkaline phosphatase (ALP) activity was elevated in treated animals.

The present data indicate that diniconazole induced a marked reduction in creatine phosphokinase (CPK) activity in the treated rats on the 15th and 30th day of experiment, while recovery to normal levels occurred after withdrawal of diniconazole. Although the values of CPK were statistically lower than that of control values, but they fell within the normal values previously reported by **Borgman *et al* (1978) and Kaneko (1989).** In this respect, **El-Sadek *et al* (1985)** mentioned that creatine phosphokinase (CPK) was reduced in treated animals with some herbicides, i.e., paraquat, sencor and dual.

In the present study, diniconazole did not induce significant changes in the plasma urea and uric acid concentrations, while creatinine concentration was markedly reduced in treated animals. Although the values of creatinine concentrations were statistically lower than those of control, but it fell within normal values of rats as reported by **Kaneko, (1989).** There findings are supported by the histopathological examination of kidney where it revealed minimal damage and degeneration in the glomeruli and tubules in treated rats. These results agree with those obtained by **Srivastava *et al* (1984), Gibson *et al* (1992) and Nasr *et al* (1996),** who mentioned that uric acid and creatinine concentrations did not alter in treated rats with some pollutants, such as N, 3,5 dichlorophenyl succinimide (NDPS), Cadmium and Phoxim.

Total protein, urea and albumin concentrations are taken together as indicators of proteins status, wherease albumin gives longer term measure of protein status. The results obtained showed insignificant changes in total protein and albumin concentrations in treated rats in with diniconazole. Such findings are agreement with the results of **Shaker *et al* (1988)** who mentioned that total protein and albumin concentrations did not alter significantly in treated animals with dimethoate.

Plasma lipids are drived from food (exogenous) or are synthesized in the body (endogenous). They are relatively insoluble in water and are carried in body fluids as soluble protein complexes known as lipoprotein. An increase in circulating levels of any lipoprotein can be caused by either excess production or decreased catabolism. Lipoprotein metabolism is of interest to clinicians concerned with diagnosis and treatment of atherosclerosis and a vascular and cardiac disease. Also, lipoprotein play an important role in cholesterol and triglycerides metabolism **(Zilva *et al* 1988).**

Results of the present work revealed that total lipids, cholesterol and triglycerides levels in the plasma of treated rats with diniconazole did not change grossly throughout the experiment and these changes returned to the normal values of control after withdrawal of diniconazole within the recovery period. Although these values were significantly different from untreated group, they fell within the normal range of rat **(Kaneko, 1989).**

In rats treated with diniconazole, the plasma glucose content decreased significantly on the 45th and 60th day of treatment. This could be attributed to calcium level which influence insulin secretion, in turn, induced glucose disappearance from plasma of treated animals **(Schlumbohm**

and Harmeyer, 1990). These results are in agreement with those recorded by **El-Sebae *et al* (1981)** who observed that significant hypoglycemia occurred in rats treated with several organophosphorus compounds.

Concerning calcium profile and calcium related regulators, the parathormone (PTH), is primary hormone regulating the concentration of extracellular calcium in mammals. Upon decreases in extracellular calcium level, PTH is released and acts on target tissues to stimulate osseous calcium mobilization and reduce renal calcium excretion, **Friedman *et al* (1996)**. PTH also activates 25 hydroxyvitamine D_3- 1 α-hydroxylase, which increases the formation of 1,25-dihydroxy vitamin D_3 and, inturn, stimulates intestinal calcium absorption. **(Brown, 1991 and Holick 1995).**

The present data revealed that diniconazole induced a marked reduction in parathormone (PTH) level after 60 day of treatment at 12 and 25 ppm, while a marked elevation occurred in PTH level on the 90th day from treatment. But after withdrawal the pesticides the level of PTH returned to the normal values within the recovery period. This may be attributed to the modulating effect of catecholamines on PTH secretion as a result of stress induced by diniconazole or due to ectopic production of PTH or PTH-like substance by non-parathyroid tissues, this known as pseudohyperparathyrodism. **(Zilva *et al* 1988).** Moreover, the level of inorganic phosphate and free ionized calcium (Ca^{2+}) did not deeply changed and the histopathology of kidney showed no deleterious effects occurred.

The results are not in agreement with those reported by **Gibson *et al* (1992)** who observed non-significant changes in parathormone level in treated rats with cadmium.

Regarding the effects of diniconazole on calcium profile, the present investigation showed that a significant decrease in total calcium and free-ionized calcium (Ca^{2+}) occurred at dose 25 ppm as well as bound calcium at doses of 12 and 25 ppm of diniconazole. It is worth to mention that although the values of these parameters are statistically lower than those of control, but they fell within the normal range **(Kaneko, 1989).**

These findings agree with those reported by **Chu *et al* (1981)** and **Mileva *et al* (1995),** who mentioned that the total calcium declined and disturbs (Ca^{2+}) homeostasis in animals treated with different substances, i.e., mono-2-ethyl hexylphthalate and fungicide GAS (N,N,N,N, tetramethyl-N,N-di (8, 15 dichloropentadeca 5, 10 dien) ethylene diamine methyl sulphate] through the investigation.

Plasma inorganic phosphate and magnasium concentrations did not alter significantly throughout the experiment. Similar results were reported by **Chu *et al* (1981)** and **Lahama *et al* (1995)** who mentioned that the magnesium (Mg^{2+}) level and inorganic phosphate levels did not alter significantly after treatment with some pesticides such as mono-2-ethyl hexyl phthalate (MEHP) and tributoxy ethyl phosphate in rats.

Regarding the internal organs weight of male rats following treatment with diniconazole, it is clear that a significant increase in the brain weight occurred after 90 days from treatment and after withdrawal the tested compound, the values of brain weights returned to the normal values.

Meanwhile, treatment with diniconazole did not change significantly the weights of liver, kidney, spleen, heart and testes. The present results are in agreement with those previously reported by **Cannon & Kimbrough (1979) and Rinner *et al* (1981)** who mentioned that the internal organs of animals treated with chlordane or 2, 4, 5 trichloro phenoxy acetic acid (2, 4, 5, T) showed non-significant differences in organs weight in treated animals.

REFERENCES

Bohuon, C. (1962). Estimation of magneisum by xylidyl blue colorimetric test. ***Anal. Chim. Acta.*** **7: 811-817.**

Brown, E.M. (1991). Extracellular Ca^{2+} sensing, regulation of parathyroid cell function, and role of Ca^{2+} and other ions as extracellular (first) messangers. ***Physiol. Rev.*** **71: 371-411.**

Borgman, R.F.; D.L. Handlin and S.F. Lightsey (1978). Serum creatine phosphokinase and amino acid concentrations in the procine stress syndrom. ***Fed. Proc.*** **37: 330.**

Cannon, S.B. and R.E. Kimbrough (1979). Short-term chlordecone toxicity in rats including Efl on reproduction pathological organ changes and their reversibility. ***Toxicol. Appl. Pharmacol.*** **47: 469-476.**

Charles, C.A.; S.P. Lucy; S.G. Cicely; W. Kichmond and C.F. Paul (1974). Enzymatic determination of total serum cholesterol. ***Clin. Chem.*** **20(4): 470-475.**

Chu, V.F.; I. A. Secours; D.C. Morine; M.S. Villeneuve and V.E. Valli (1981). Sub-acute and sub-chronic toxicity of mono-2-ethyl hexyl phthalate in the rat. ***Arch. Environ. Contam. Toxicol.*** **10: 271-280.**

Coulombe, J.J. and L. Farreau (1963). Anew simple semi-micro method for colourimetric determination of urea. ***Clin. Chem.*** **9: 102-108.**

Darham, B. and P. Trinder (1972). An improved colour reagent for determination of blood glucose by the oxidase system. ***Analyst.*** **97: 142-145.**

Dixon, W.J. and J.F. Massay (1957). Introduction to Statistical Analysis. 2nd (Ed.) McGraw Hill Book Co. Inc., New York.

Drupt, F. (1974). Determination of human serum albumin. ***Farm. Biol.*** **9: 222.**

El-Maassarawy, A.D. (1996). ***Effect of Some Heavy Metals on Thyroid, Kidney and Testis Function.*** **pp. 67-74.** Ph.D. Thesis. Fac. of Veterinary Medicine, Cairo University.

El-Sadek S.E.; S.S. Ibrahim; S.A. Abdel-Salam and S.A. Regel (1985). Effect of three herbicides on some enzyme activity and haematological changes in rats. ***Vet. Med.*** **J. 33(1): 123-133.**

El-Sebae, A.H.; E.E. Enan; S.A. Soliman; S. El-Fiki and E. Khamees (1981). Biochemical effects of some organophosphorus insecticides on new targets in white rats. ***(Rattus rattus norvegicus). J. Environ. Sci. Health*** **16(4): 475-492.**

Fossati, F. and P. Prencipe (1988). Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. ***Clin. Chem.*** **28(10): 2077-2080.**

Friedman, P.A.; B.A. Gutermorsh; S.M. Kennedy and F.A. Gesek (1996). Parathyroid hormones stimulation of calcium transport is mediated by dual signaling mechanisms involving protein kinase A and protein kinase C. ***Endocrinology,*** **37(1): 13-20.**

Frings, C. and R.T. Dum (1970). Colourimetric method for determination of total serum lipids based on sulphophosphoxaminthin reaction. *Amr. J. Clin. Path.* 53: 89-91.

Gibson, N.S.; S. Chaudhry; A. Brock; A.B. Sickles; B. Patel; R. Hegsted; S. Johnston; D. Peterson and M. Bhattachargye (1992). Cadmium effects on bone marrow metabolism. Accelerated resorption in ovariectomized aged beagles. *Toxicol. Appl. Pharmacol.* 113: 274-283.

Gojmerac T.; B. Kartal; M. Zuric and M. Mitak (1995). Serum biochemical and histopathological changes related to the hepatic function in pigs following atrazine treatment. *J. Appl. Toxicol.* 15(3): 233-236.

Hawker, C.D. and F.P. Dibella (1980). Radio-immunoassay for intact and carboxyl terminal parathyroid hormone, clinical interpretation and diagnostic significance. *Ann. Clin. Lab. Sci.* 10: 76-88.

Hausdman, T.U.; W.R. Helger and W. Gress (1967). Optimal conditions for the determination of serum alkaline phosphatase by a new kinetic method. *Clinica Chimica Acta* 15: 241-245.

Holick M.F. (1995). Vitamin D; Photobiology, metabolism and clinical applications. In: De Groot LJ, Besser M; Burger H.G.; Jameson JL; Loriaux DL; Marshal J.C., Odella W.D.; Potts J.r.J, T, Rubenstein A.H. (eds). *Endocr nology*. 990-1014. Saunders, Philadelphia.

Husdan, H. and A. Rapoport (1968). Estimation of creatinine by Jaffe reaction. A comparsion of methods. *Clin. Chem.* 14: 222-238.

Kaneko J.J. (1989). Clinical Biochemistry of Domestic Animal. 4th Ed., pp. 892-897. Academic Press Inc. Harcourt Brace. Jovanovich Publishers, New York.

Lahama, S.L.; G.W. Long and B.R. Broxup (1995). Subchronic oral toxicity of tri-butoxy phosphate in the sprague Dawley rat. *Arch. Environ. Health* 40(1). 12-17.

Mileva, E.; A. Krislev; N. Stoichev; A. Nikolov and N. Nikolov (1995). Disturbances in rat smooth-muscle induced by a substance with fungicide action. *J. Appl. Toxicol.*, 15(3): 219-222.

Morin, L.G. (1977). A colorimetric method for the quantitative determination of serum creatine kinase. *Clin Chem.* 23: 464 - 471.

Nasr M.Y.; M.M. Nassif and F.M. Fouad (1996). Some of the clinico-biochemical effects of organophosphorus insecticides (Phoxim) in rams. *Vet. Med. J. Giza:* 44(2): 331-338.

Puerro M. and M.A. Alexandre (1995). Induction of acute hypocalcemia in rat. *Vet. Human Toxicol.* 37(2): 116-117.

Reitman, S. and S. Frankel (1957). A colourimetric method of the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Amr. J. Clin. Pathol.*, 28: 53-56.

Rinner G.; B. Herforth; J.M. Gokel; G. Goerz and M.L. Luderschmidt (1981). Subchronic toxicity studies on pentachloronitrosobenzene (PCNO) in female rats. *Ecotoxicol. & Environ. Safety*, 5: 281-290.

Robertson W.G. and R.W. Marshall (1979). Calcium measurement in serum and plasma total and ionized Crc. *Crit. Ren. Clin. Lab. Sci.* 13: 271-304.

Schalm, O.W. (1986). *Veterinary Haematology.* 4th (Ed)., pp. 21-86. *Lea &* Febigen, Philadelphia.

Schlumbohm C. and J. Harmeyer (1990). Hypocalcemia reduces rate of disappearance of glucose from plasma. *J. Vet. Med.*, 37: 285-293.

Shaker N.; G.A. Hassan; F.D. El-Nouty; A.S. Abo El-Ezz and G.A. Abd-Allah (1988). In vivo chronic effect of dimethoate and deltamethrin on rabbits. *J. Environ. Sci. Health*, B23 (4): 387-399.

Snedecor, G.W. and W.G. Cochran (1980). Statistical Methods. 7th (Ed).pp. 82-99. State University Press. Ames., Iowa.

Srivastava P.K.; V.K.Chaudhary; S.S. Chauhan; V.K. Srivastava and U.K. Misra (1984). Biochemical and pathological effects of fly ash on lung, liver and blood of rats. *Arch. Environ. Contam. & Toxicol.* 13: 441-452.

Triwedi R.C.; R. Linda; B. Elieen and M.S. Stong (1978). New enzymatic method for serum uric acid at 500 nm. *Clin. Chem.*, 24(11): 1908-1911.

Weill C.S. (1952). Tables for convenient calculation of medium affective dose. (LD_{50} and ED_{50}) and instruction in their use. *Biometrics* 8: 249-263.

Weichselbaum, T.E. (1946). Determination of protein in small amounts of blood serum and plasma. *Am. J. Clin. Pathol.* 16: 40-44.

Zidan, A.A.; M.I. Hussein and S.M. Dahrog (1991): Effect of certain insecticides on some biochemical aspects of blood of male mice by oral administration. *4th Arab Cong. of Plant Protection. Cairo, p.* 184.

Zilva J.F.; P.R. Pannall and P.D. Mayne (1988). *Clinical Chemistry in Diagnosis and Treatment.* 5th (Ed.,) pp. 172-199. Edward Arnold, London.

مجلة اتحاد الجامعات العربية للدراسات والبحوث الزراعية ، جامعة عين شمس ، القاهرة ، ١١(٢) ، ٩١٣ - ٩٣٣ ، ٢٠٠٣

تأثير الدينيكونازول علي نظام التمثيل في الفئران البيضاء

[٦٥]

زيدان هندي[١] - مصطفي الهراوي[٢] - سمير غنيم[٣] - مجدي محمد السيد[٢]

١- كلية الزراعة - جامعة عين شمس - شبرا الخيمة - القاهرة - مصر

٢- المعمل المركزي للمبيدات - وزارة الزراعة - الدقى - جيزة - مصر

٣- كلية الزراعة - جامعة قناة السويس - الإسماعيلية - مصر

أجريت هذه الدراسة لتقييم السمية تحت المزمنة للمبيد الفطري داينيكونازول في ذكور الفئران البيضاء . لقد سبب الداينيكونازول نقص معنوي في نشاط انزيم أسبرتات أمينوترانسفيريز علي امتداد ٩٠ يوما من المعاملة ، بينما لم يحدث المبيد أية تغيرات في انزيم الأنين أمينوترانسفيريز خلال فترة التجربة . حدثت زيادة ملحوظة في انزيم فوسفاتيز القلوي في بلازما الحيوانات المعاملة بالداينيكونازول وقد عاد النشاط الانزيمي لمستواه الطبيعي بعد فترة الشفاء . لقد حدث نقص معنوي في مستوي

الكرياتين فوسفو كينيز بعد ١٥ يوما من المعاملة بينما حدثت زيادة ملحوظة بعد ٣٠ يوما واقتربت من المستويات العادية خلال فترة الشفاء ٠ لم تحدث معاملات الداينيكونازول أي تغير في مستويات اليوريا وحمض اليوريك والبروتين الكلي والألبيومين والماغنسيوم بينما انخفضت مستويات الجلوكوز والكرياتين في الفئران المعاملة بعد ٩٠ ، ٤٥ يوما من المعاملة علي التوالي ٠

لقد أختلفت مستويات البلازما والليبيدات الكلية والكوليستيرول الكلي والجلسريدات الثلاثية خلال فترة التجربة ٠ أظهرت المعاملة بالداينيكونازول لمدة ٩٠ يوما تأثير سام قليل علي مستوي هورمون الباراثورمون ونظام الكالسيوم ومستوي الفوسفور غير العضوي ٠ حفز الداينيكونازول زيادة معنوية في أوزان الجسم والمخ بينما لم تتغير أوزان الكبد والرئتان والقلب والكلي والطحال والخصيات خلال فترة التجربة ٠ لقد حدث تلف متوسط في أنسجة الكلي من جراء المعاملة بالداينيكونازول ٠

تحكيم: أ.د عبد السلام قنصوة
أ.د العيداروس أحمد جمعه