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THE PROTECTIVE ROLE OF VITAMIN E AND SELENIUM AGAINST THE HARMFUL EFFECTS OF DINICONAZOLE FUNGICIDE IN MALE ALBINO RATS

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ABSTRACT

The present study conducted to evaluate the toxic effects of dinconazole fungicide at 22 mg/kg B.W (1/10 of oral LD_{50} in male albino rats) and the protective role of vitamin E and selenium (Se) as antioxidants against the adverse effects of tested fungicide. Dinconazole treatments produced erythropenia, leucopenia and nephrotoxicity in addition to hepatotoxicity associated with a marked elevation in alanine aminotransferase (ALT) activity and the rate of lipid peroxidation in hepatic tissues of dinconazole- treated rats. Hypercholesterolemia accompanied by hypotriglycerdemia and a reduction in high density lipoprotein (HDL-C) as well as hypothyroidism were also noted in dinconazole- treated rats. Oral administration of vitamin E or selenium (Se) pre-treated with dinconazole resulted in a reduction of hematotoxicity and hepatotoxicity and hepatic MDA production, which is the index of lipid peroxidation. Also, all significant changes seem in lipidogram parameters disappeared and their values resumed to control level except for creatinine level and Thyroid hormones (T4 & T3) which did not influenced by antioxidant supply did not influence by antioxidants supply.

Key words: Vitamin E – Selenium – Diniconazole – Fungicide.

INTRODUCTION

A role of free radicals has been proposed in the toxicity of numerous chemicals and in the pathogenesis of many diseases. In addition, some clearly deleterious phenomena such as lipid peroxidation also occur at the point of cell death and membrane lysis [Kehrer, 1993].

The free radical, including oxyradical can react with large variety of biomolecules and fundamental lessions associated with oxyradicals include oxidation of membrane lipids, proteins and nucleic acids and altered cellular redox status [Ames, 1989]. An enormous amount of data has been published in recent years demonstrating vitamin E (α-Tocopherol) or selenium have defines role against toxic-free radicals. Vitamin E (α-Tocopherol) is physiological scaveingers of free oxygen radicals (FOR) but an additional mode of action based on stabilization of membranes may also be involved, since it is incorporated into the lipid bilayer of various membranes [Paker, 1991]. In addition, vitamin E is a potent antioxidant, by interacts with lipid pertly radicals and form relatively unreactive tocopheroxyl radicals and lipid hydroperoxides, therefore, α-Tocopherol inhibits lipid peroxidation [Mickle and Weisel, 1993]. Furthermore, vitamin E supplementation is able to correct cellular depletion of vitamin E and reduce membrane lipid peroxidation [Rosen and Toeller, 1999]. Numan *et al.* (1990) found that the vitamin E administration prevented the induction of hepatic lipid peroxidation by endrin insecticide.

In respect of selenium (Se), it is an essential trace element in both human and animals. The Se is an integral part of the glutathione peroxidase enzymes. Therefore, Se is regarded as a component of the defense mechanism against the harmful effects of free oxygen radicals (FOR) and lipid peroxidation [Thompson, 1984, Hawkes *et al.*, 1985 and Donaldson, 1994].

The present study aimed to clarify the positive role of vitamin E and selenium in protecting treated male albino rats against the adverse effects of diniconazole fungicide. The actions of these antioxidants were investigated on the haemogram, clinical biochemistry markers, extent of hepatic lipid peroxidation, lipidogram and Thyroid hormones in blood rats.

MATERIAL AND METHODS

Chemicals:

Commercial formulation of diniconazole fungicide [(E)-1- (2, 4- dichlorophenyl - 4,4 dimethyl-2- (1,2,4 traizole-1-yl) 1-penten-3-ol), was supplied by Sumitomo Chemical Co. Japan. The vitamin E (D-α-Tocopherol) and selenium (sodium selenate) were purchased from Merck Co. Germany. Also, 2-Thiobarbituric acid (TBA) and 1, 1, 3, 3-tetramethoxy propane were obtained from Sigma Chemical Co. (St. Lowis, M) and Merck Co. Germany, respectively. All other chemicals were of reagent grade.

Experimental animals:

Male albino rats (Wister Strain) weighted approximately 140-160 g, were purchased from Organization of Biological Products and Vaccines (Helwan Farm). The rats were kept in plastic cages under hygienic conditions and fed on standard diet and drinking tap water *ad -libitium.*

Experimental procedure:

After an acclimatization of 2 weeks, the animals were grouped as follow: the first group of animals received orally distilled water and served as control group. The second group received the diniconazole alone (designated as Dn) .The third and fourth groups received vitamin E and selenium pre-Treated with diniconazole (designated as E+ Dn and Se + Dn, respectively). Diniconazole administration was done through oral at dosage of 22 mg /Kg B.W (1/10 of oral LD_{50}) , whereas vitamin E and selenium were supplied to rats at dosages of 12 mg and 50 µg, respectively, for 28 day and which calculated according to the method of Paget and Barnes (1964). A vitamin E or selenium alone group was not assigned because the vitamin E or selenium dosages used were of low toxicity and represented the Recommended Daily Allowance (RDA) [National Research Council, (NRC), 1989]. The blood samples were taken at 7, 14, 21 and 28 day. Procedures were in accordance with the OECD Guideline of Testing Chemicals (No.407, 1995).

Blood Sampling:

Fasting blood samples were taken via orbital sinus vein at 7, 14, 21 and 28 day after beginning of the administration (Ston, 1939). The blood samples were collected in EDTA-K_2 anticoagulant tubes for

haematological investigation and in hepariazed or in plain tubes for plasma or serum clinical biochemistry (Schalm, 1986).

Biochemical analysis:

Plasma enzyme activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), as well as the serum total protein and albumin were determined according to the methods of Reitman and Frankel (1957); Hausdman *et al.* (1987) Weishselbaum, (1946) and Drupt (1974), respectively. The globulins calculated from subtracting the albumin from serum total protein. Also, kidney function parameters as urea and creatinine were measured by using the method of Coulombe and Farreaus (1963) and Husdan and Raport (1968), respectively. In addition, lipidogram, included Triglyceride (TG), Total cholesterol (TC), low denisty lipoprotein- cholesterol (LDL-C) and high denisty lipoprotein-cholesterol (HDL-C), these parameters were determined according to the methods of Bucolo and David (1973), Trinder (1967) and Wieland and Seidel (1983), respectively. All these parameters were determined by using Diagnostic kits.

Determination of lipid peroxidation in hepatic tissue:

A lipid peroxidation was determined in whole homogenate of the liver of rats treated with diniconazole alone or antioxidant plus diniconazole and control animals according to the method of Ohkawa *et al.* (1979). Malondialdehyede (MDA) occurs in lipid peroxidation and this is measured after incubation at 95°C with thiobarbituric acid in aerobic condition (pH, 3.4). The pink colour formed in these reactions is measured in the spectrophotometer at 532 n m to measure MDA.

Hormonal analysis:

The total Thyroxine (T4) and total triiodothyronine (T3) were determined by using the Coat-A- Count Procedure, which is a solid radioimmuno assay (RIA). It was carried out according to the method adopted by Britton *et al.* (1975).

Statistical analysis:

Data are presented as the mean of five samples with their standard error. Significant of the values obtained were tested using Student "T-test" [Gad and Weil, 1989].

RESULTS

Data of the haematotoxicity of diniconazole alone at dosage of 22 mg/kg B.wt in male albino rats following repeated exposure for 28 day and after supplementation with vitamin E or selenium for the same period are tabulated in Table (1). A significant increase in the haemoglobin concentration (Hb) and mean corpuscular haemoglobin concentration (MCHC) was observed in dinconazole-treated rats after 7 and 21 days of treatment. However, these parameters restored to the control values after supplementation with Vit. E or selenium (Se) pre-exposure to diniconazole. Also, a significant elevation in the packed cell volume (PCV) was noted in rats following exposure to diniconazole at 21 and 28 days but supplemented these animals with Vit. E or selenium resulted in approached the values of PCV to the normal level of control group. In addition, the current study has shown that diniconazole treatment induced leucopenia, neutropenia and lymphopenia at the end of the experimental period. (28 days). Meanwhile, the rats when supplemented with the Vit. E or Se before treatment by diniconazole showed insignificant differences in the total leukocytes, neutrophils and lymphocytes counts between antioxidants-supplemented rats and control individuals. A significant enhancement in the activity of ALT was observed after 21 and 28 days of treatment with diniconazole and a significant decrease in the activity of AST was observed, in diniconazole- treated rats. However, supplementation rats with Vit. E or Se before exposure to diniconazole showed a marked decrease in the activity of ALT to the values of control group.

A remarkable reduction of plasma ALP activity was observed in the diniconazole-exposed rats at 21 and 28 days. Meanwhile, supplementation of rats with Se showed modulation effect on the activity of ALP, which restored to the control level. Also, administration of diniconazole at 22-mg/kg b.wt resulted in a significant elevation in the serum total protein (TP) and globulin concentrations. Supplementation rats with Vit. E or Se before exposure to diniconazle resumed the values of T.P and globulins to the control levels.

Malondialdehyde (MDA) concentration in whole hepatic homogenate of diniconazole-treated rats was significantly higher than that of the control group at the end of the experimental period. (28 days). But supplemented rats with Vit. E together with diniconazole

Table (1): Effect of vitamin E and selenium (Se) supplementation on haemogram and differential of leukocytes in diniconazole- exposed rats.

Periods	7 day				14 day				21 day				28 day			
Parameters	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din
RBCS $x10^6$/ul	6.305 ± 0.308	7.128 ± 0.240	7.036 ± 0.376	6.818 ± 0.604	6.798 ± 0.31	7.574 ± 0.513	6.208 ± 0.164	5.848 ± 0.565	6.478 ± 0.407	6.462 ± 0.152	6.182 ± 0.382	8.018 ± 0.341	5.798 ± 0.488	5.550 ± 0.394	6.284 ± 0.275	7.558 ± 0.845
Hb g/dl	9.75 ± 0.55	12.48 ± 0.429**	11.930 ± 0.707	11.489 ± 0.923	9.346 ± 0.624	10.877 ± 1.110	11.060 ± 0.731	9.992 ± 1.060	10.01 ± 0.296	12.676 ± 0.603***	10.416 ± 0.395	12.954 ± 0.351	10.108 ± 0.48	9.041 ± 1.046	10.015 ± 0.564	10.126 ± 0.567
PCV %	46.00 ± 2.07	50.40 ± 2.014	47.8 ± 1.241	50.6 ± 5.085	45.40 ± 2.94	48.40 ± 3.218	45.40 ± 2.694	41.8 ± 2.353	42.40 ± 1.568	50.40 ± 1.60**	47.60 ± 1.288	43.2 ± 1.562	44.98 ± 0.67	49.23 ± 0.52**	45.20 ± 1.907	40.80 ± 1.881
MCV ft	74.88 ± 4.941	69.685 ± 2.305	68.698 ± 4.163	74.136 ± 4.165	76.52 ± 5.04	64.761 ± 6.150	73.057 ± 3.442	73.005 ± 4.337	73.46 ± 5.36	78.049 ± 2.194	62.114 ± 1.631	54.228 ± 2.901	76.55 ± 5.08	79.018 ± 6.951	66.927 ± 3.617	66.461 ± 3.572
MCH pg	14.97 ± 1.71	18.258 ± 0.697	17.027 ± 0.921	14.899 ± 1.532	15.65 ± 1.67	14.567 ± 1.836	17.778 ± 0.885	17.417 ± 1.717	14.786 ± 1.766	17.21 ± 0.85	15.976 ± 1.138	16.293 ± 0.917	15.57 ± 1.66	16.423 ± 1.949	16.062 ± 1.116	14.100 ± 1.710
MCHC %	20.81 ± 0.942	22.128 ± 1.16	22.270 ± 1.72	21.157 ± 1.772	20.414 ± 1.362	22.274 ± 1.202	24.345 ± 0.541	23.8.6 ± 1.729	21.420 ± 0.79	25.103 ± 0.419**	22.918 ± 1.802	22.44 ± 1.064	18.871 ± 1.569	20.674 ± 1.179	19.920 ± 2.009	25.011 ± 1.704
WBCs $x10^3$/µl	11.47 ± 0.33	9.470 ± 0.857	11.26 ± 0.931	10.21 ± 0.897	11.650 ± 0.274	10.61 + 0.99	10.03 ± 0.688	10.10 ± 0.998	11.930 ± 0.136	10.19 ± 0.808	10.52 ± 1.820	7.98 ± 0.403	11.94 ± 1.36	7.083 ± 0.346**	9.480 ± 0.918	8.18 ± 0.748
Neutrophil $x10^3$/µl	1.770 ± 0.208	1.409 ± 0.075	2.724 ± .359	2.351 ± 0.381	1.647 ± 0.287	1.660 ± 0.733	1.717 ± 0.160	1.1003 ± 0.151	1.493 ± 0.245	1.202 ± 0.051	1.811 ± 0.276	1.474 ± 0.110	1.381 ± 0.044	0.915 ± 0.117*	1.301 ± 0.105	1.376 ± 0.176
Lyphocytes $x10^3$/µl	9.069 ± 0.876	8.015 ± 0.089	7.842 ± 0.953	7.927 ± 0.630	9.952 ± 0.254	9.234 ± 0.812	8.574 ± 0.107	10.136 ± 0.750	9.269 ± 0.432	7.96 ± 0.47	9.467 ± 1.335	8.946 ± 1.163	9.380 ± 1.382	6.128 ± 0.283*	12.777 ± 0.935	7.699 ± 0.261

Din: diniconazole fungicide RBC: Red Blood Cells Hb: Haemoglobin PCV: Packed Cell Volume
MCV: Mean Corpuscular Volume MCH: Mean Corpuscular Haemoglobin MCHC: Mean Corpuscular Haemoglobin concentration
WBCs: White Blood Cells
*Data represented the mean ± SE.M (n=5)
*Asterisks indicated that values are different from the control group at *P< 0.05 **P< 0.01 ***P < 0.001

Table (2): Effect of vitamin E and selenium (Se) supplementation on biochemical biomarkers in dinconazole-exposed rats

Periods	7 day				14 day				21 day				28 day			
Parameter	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din
ALT IU / l	37.94 ± 5.10	36.91 ± 5.70	21.851 ± 1.473*	27.581 ± 2.576	32.310 ± 6.090	50.65 ± 5.70	30.555 ± 0.765	27.047 ± 2.60	33.254 ± 5.435	50.51 ± 4.6*	45.553 ± 9.001	28.555 ± 3.289	37.20 ± 0.21	50.647 ± 3.98*	42.536 ± 2.111	28.886 ± 3.579
AST IU /l	102.082 ± 5.910	89.166 ± 5.796	119.583 ± 11.936	113.194 ± 6.492	96.288 ± 7.109	108.11 ± 7.75	95.395 ± 9.97	60.832 ± 4.146	94.542 ± 5.068	110.416 ± 7.963	103.749 ± 3.254	89.930 ± 4.075	105.5 ± 7.7	79.721 ± 8.019*	120.032 ± 6.838	121.006 ± 8.531
ALP IU /d l	27.614 ± 5.465	36.329 ± 2.935	27.334 ± 1.30	18.930 ± 1.411	31.237 ± 2.427	10.916 ± 1.621***	27.889 ± 1.24	26.317 ± 1.257	29.128 ± 2.397	10.549 ± 2.262***	21.865 ± 0.524*	22.893 ± 1.617	29.311 ± 2.940	22.339* ± 0.530	15.213 ± 1.487**	26.47 ± 2.109
S . P g / dl	6.462 ± 0.269	6.356 ± 0.435	6.215 ± 0.553	5.553 ± 0.604	6.205 ± 0.432	5.362 ± 0.539	5.759 ± 0.345	5.821 ± 0.395	5.852 ± 0.349	5.498 ± 0.158	6.781 ± 0.631	6.054 ± 0.419	5.085 ± 0.276	7.931 ± 038**	5.238 ± 0.253	5.546 ± 0.452
Albumin g/dl	3.722 ± 0.431	2.678 ± 0.438	2.444 ± 0.282	4.132 ± 0.104	4.211 ± 0.221	2.88 ± 0.62	4.752 ± 0.159	4.709 ± 0.195	3.987 ± 0.300	3.938 ± 0.150	2.648 ± 0.380	3.941 ± 0.437	3.586 ± 0.294	2.847 ± 0.267	2.64 ± 0.414	4.006 ± 0.262
Globulin g/dl	2.842 ± 0.670	3.678 ± 0.256	1.614 ± 0.282	2.252 ± 0.069	2.415 ± 0.357	4.678 ± 0.178	1.474 ± 0.275	1.287 ± 0.170	2.082 ± 0.246	2.938 ± 0.842	3.482 ± 0.264	2.293 ± 0.576	2.544 ± 0.471	5.074 ± 0.272***	3.013 ± 0.155	1.717 ± 0.304

ALT : Alanine amino transferase AST : Aspatate amino transferase
ALP : Alkaline phosphatase S.P : Serum protein
*Data represented the mean ± SE.M (n=5)
*Asterisks indicated that values are different from the control group at *P< 0.05 **P< 0.01 ***P < 0.001

showed a significant decrease in MDA level in comparison with control rats. Also, supplemented animals with Se the level of MDA brought to normal level and had no significant variations from control groups [Table, 3]. There was a significant increase in the urea level in diniconazole-treated rats at 7 , 21 and 28 days, but supplemented rats with Vit. E or Se pre-exposure to diniconazle, had normal level of urea when compared with control rats. Moreover, a significant rise in the creatinine level was detected in diniconazole-treated rats after 21 and 28 days of treatment and supplemented rats with Vit. E diminished the creatinine level to the normal level of untreated rats, whereas the Se supplementation did not ameliorate this disorder as shown in Table (4). The results presented in Table (5) indicated that the concentration of Triglycerides (TG) in the plasma of rats following exposure to diniconazole was lowered than that in the control rats and supplemented individuals with Vit. E or Se, returned TG values to the normal level of untreated animals over the experimental period. In contrast, hypercholestrolemia was detected in diniconazole-treated rats for 21 and 28 days of treatment but supplemented rats with Vit.E or Se approached the cholesterol concentrationto the normal level.

Also, the level of low-denisty lipoprotein cholesterol (LDL-C) was elevated markedly in rats following exposure to diniconazole after 21 and 28 days of exposure. With respect to the level of high denisty lipoprotein cholesterol (HDL-C), there was a marked decrease in the level of HDL-C in diniconazole-treated rats during the experimental period (28 days). But, the administration of diniconazole with selected antioxidants (i.e., Vit. E or Se) exhibited improvable effect of these antioxidants on the hypotriglyceridaemia and hypercholesterolaemia in diniconazole-treated rats, where the values of these parameters restored to the values of control animals, except of the HDL-C level in Se - Supplemented rats was below the control values after 14 day of treatment (Table, 5).

In addition, a remarkable reduction in the concentration of Thyroxine (T4) and Triiodothyronine (T3) was observed in diniconazole-treated rats. Administration of Vit. E or Se to rats before exposure to diniconazole had no significant effect on the depression of thyroid hormones (Table 6).

Table (3): Effect of Vitamin E and selenium (Se) supplementation on hepatic lipid peroxidation in diniconazole - exposed rats.

Treatment period (28 day)								
Parameter	Cont.		Din		E+Din		Se+Din	
Malondialdehyde (MDA) n mol/gm tissue of liver	355.38 ± 8.875	100.0%	411.90 ± 9.36**	115.902 %	195.94 ± 5.32***	55.0%	397.101 ± 17.209	111.73%

Table (4): Effect of Vitamin E and selenium (Se) on kidney function parameters in diniconazole -exposed rats.

Period	7 day				14 day				21 day				28 day			
Parameter	**Cont.**	**Din**	**E+Din**	**Se+Din**	**Cont**	**Din**	**E+Din**	**Se+Din**	**Cont.**	**Din**	**E+Din**	**Se+Din**	**Cont.**	**Din**	**E+Din**	**Se+Din**
Urea mg/dl	16.876 ± 1.426	26.44 ± 0.97***	20.980 ± 2.542	26.059 ± 1.437**	18.4112 ± 1.595	16.46 ± 0.860	21.031 ± 2.61	18.554 ± 1.382	17.883 ± 1.121	22.748 ± 0.842**	23.84 ± 2.454	20.864 ± 1.418	17.677 ± 1.910	24.11 ± 1.34**	18.954 ± 1.123	14.051 ± 2.375
Creatinine mg/l	6.180 ± 0..509	5.172 ± 0.230	6.250 ± 0.40	6.120 ± 0.29	5.70 ± 0.460	5.829 ± 0.57	4.830 ± 0.81	7.120 ± 0.24*	5.80 ± 0.30	9.00 ± 0.20**	6.870 ± 0.78	10.626 ± 0.63***	6.05 ± 0.46	11.35 ± 0.58***	4.84 ± 0.19*	7.42 ± 0.11*

Din: diniconazole fungicide

***Data represented the mean ± SE.M (n=5)**

***Asterisks indicated that values are different from the control group at *P< 0.05 **P< 0.01 ***P < 0.001**

Table (5) Effect of vitamin E and selenium (Se) supplementation on lipidogram in diniconazole-exposed rats.

Periods	7 day				14 day				21 day				28 day			
Parameter	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din	Cont	Din	E+Din	Se+Din
Tg mg/dl	39.209 ± 1.977	24.189 ± 3.917**	49.300 ± 4.555	45.28 ± 9.236	37.657 ± 3.617	29.406 ± 2.590*	42.687 ± 4.271	28.932 ± 4.239	37.66 ± 1.77	28.30 ± 1.702*	59.762 ± 9.281	29.468 ± 3.809	36.448 ± 1.288	18.339 ± 1.94**	36.099 ± 3.054	35.571 ± 1.983
TCh mg/dl	48.768 ± 4.16	51.87 ± 5.58	57.357 ± 2.014	40.339 ± 1.472	48.648 ± 3.295	55.104 ± 4.070	42.266 ± 3.970	55.705 ± 5.410	48.167 ± 4.715	60.360 ± 0.300**	39.189 ± 3.168	42.516 ± 3.465	44.636 ± 3.477	61.411 ± 3.280***	40.277 ± 0.711	37.687 ± 4.222
LDL-C mg/dl	33.49 ± 2.57	28.25 ± 1.40	29.725 ± 4.423	38.29 ± 3.050	32.04 ± 1.66	46.419 ± 13.20	34.99 ± 5.09	35.10 ± 3.40	34.680 ± 3.03	58.17 ± 0.80**	21.302 ± 1.869	38.79 ± 1.222	33.54 ± 0.432	67.04 ± 3.600***	29.302 ± 1.870	30.36 ± 0.692
HDL-C mg/dl	21.472 ± 2.023	13.78 ± 0.654***	23.157 ± 1.313	24.43 ± 0.556	21.682 ± 1.952	16.45 ± 0.313*	21.28 ± 1.606	15.855 ± 0.630*	22.554 ± 1.241	15.197 ± 1.63**	20.79 ± 3.008	18.79 ± 2.453	21.273 ± 1.334	14.56 ± 0.432*	19.372 ± 0.990	20.317 ± 1.077

TG: Triglyceride T.Chol: Total cholesterol
LDL-C: Low density lipoprotein-cholesterol HDL-C: High density lipoprotein-cholesterol

Table (6): Effect of Vitamin E and selenium (Se) on Thyroid hormones (T4 &T3) in diniconazole -exposed rats.

Period	Treatment period (28 day)			
Parameter	Cont.	Din	E+Din	Se+Din
Thyroxine (T4) µg/dl	1.915±0.191	1.049±0.092**	1.203±0.091**	1.269±0.140*

*Data represented the mean ± SE.M (n=5)
* Asterisks indicated that values are different from the control group at *P< 0.05 **P< 0.01 ***P < 0.001

DISSCUSION

Certain pesticides, drugs including paracetamol, hepatoxins as carbon tetrachloride are known to be exogenous generators of superoxide ($^{\cdot}O_2$) [Duthie *et al.*, 1989]. The antioxidants must be present at the time of exposure to the promoter of oxidative stress, in order to produce protection. Since this is the time that free radical production is enhanced, therefore, treatment with antioxidants at other times may have no effect or may even have a promoting effect (Probably) through some mechanisms unrelated to free radicals (Thompson *et al.*, 1991). A large number of antioxidants either enzymatic or non-enzymatic systems, are controling *in vivo* the action of the free radicals. Among the non-enzymatic antioxidants, the vitamin E (α-Tocopherol) and trace element selenium (S) are effective against the free-radicals actions (Paker, 1991 and Reilly, 1996).

Rats administrated diniconazole alone showed immuno-suppressive effect, including, leucopenia, neutropenia and lymphopenia but supplemented rats with Vit.E or Se before treatment with diniconazole, resulted in immunostimulation in antioxidant-supplemented rats. As for, the other haematological disorders in diniconazole-treated rats, were disappeared in supplemented rats with tested antioxidants pre-exposure to diniconazole.

The previous findings suggest that Vit.E deficiency developed severe anemia because of the bone marrow shows ineffective erythropoiesis and red cells are highly sensitive to hemolysis by H_2O_2. However, erythrocytes GSHPX correlate directly with selenium in the erythrocytes. Therefore, in the erythrocytes GSHPX protects haemoglobin from oxidation by hydrogen peroxide (H_2O_2). [Jain, 1993]. Also, selenium at certain level may counteract the immunosuppressive effect of xeinobiotic toxicity and resulted in immunostimulation effect (Methenitau *et al.*, 1996). However, stimulation of hematopoisis in bone marrow of rats were exposed to diniconazole after Vit. E supplementation might be a physiological response to the leukopenia in diniconazole-treated rats [Mankenzei *et al.*, 1990 and Irons, 1991].

The mechanism for the ability of Vit. E to enhance the immunoresponse in Vit. E supplemented rats was proved through the present study. This may be attributed to the Vit. E supplementation had stimulation effect on the bone marrow, which responsible for

proliferation of blood cells in living organisms. Also, Herberal *et al.* (1988) reported that Vitamin E causes a significant improved in cellular and humoral immune response. In general, vitamin E deficiency and low tissue vitamin E content enhance the component of the inflammatory response and suppress components of the immune response (Meydani *et al.*, 1992 and Makindom, 1995). It is interesting to note that the vitamin E has been suggested to act as antioxidant not only via its ability to scavenge the free radicals and also by preventing the accumulation of neutrophils (neutropenia) within injury tissue (i.e. ischaemic tissue) [Formigli *et al.*, 1997].

Our findings indicated that the major site of diniconazole toxicity was the cytosol of hepatocytes and this give rise to release the cytosolic ALT readily than AST, which held inside mitochondria of hepatocytes. Moreover, it was reported that higher plasma activities of ALT and AST were found to be response to oxidative stress. [Davy *et al.*, 1985]. When rats exposed to diniconazole following supplementation with Vit. E or selenium had normal level of ALT activity. This suggests that administration of the Vit. E or Se together with diniconazole reversed the hepatotoxicity caused by diniconazole alone. This probably due to the protective effect of Vit. E and Se by increasing the catabolism (plasma clearance) of enzymes in circulating blood and/or reducing the leakage of liver enzymes from hepatocyte membranes (Zilva *et al.*, 1988).

No recovery observed in the activity of ALP of vitamin E-supplementation rats. This could be attributed to inadequate supply of Vit. E and/or shortend the duration of supplementation.

Furthermore, hyperproteinemia and hyperglobulinemia were observed in diniconazole-exposed rats where, the immunoglobulin increased as a consequence of inflammatory process (Roth, 1995). However, Co-administration of selenium or vitamin E with diniconazole caused reduction in the level of total protein and globulines to the levels of normal rats. This may be contributed to the anti-flammatory effect of such antioxidants. (Moore *et al.*, 1992 and Werner *et al.*, 1994). A significant elevation in the MDA level in the whole liver homogenate of diniconazole-treated rats was observed.

Increase of Thiobarbituric Acid Reactive Substance (TBARS) level as the amount of malondialdehyde (MDA) in hepatic tissue, indicated the presence of oxidative injury in the hepatocytes of diniconazole-treated rats. Therefore, MDA is considered a major

degradation product of endogenous lipid peroxidation and measurement of MDA concentration is generally accepted as a marker as for assessing the extent of lipid peroxidation *in vivo* [Kishida *et al.*, 1993 and Raharjo *et al.*, 1993].

Our results suggest that Vit. E and Se supplementation lessened the hepatic lipid peroxidation, which produced by diniconazole-treatment when compared with untreated rats. In addition, supplementation with Vit. E exhibited greater protection against hepatotoxicity than Se supplementation, this reflected in reducing of MDA level in Vit. E Supplemented rats than normal rats. This may be due to a chroman-ring of α-Tocopherol, which is the active antioxidant part of the molecule of α-Tocopherol and responsible for inhibition of lipid peroxidation, via incorporation into the lipid bilayers of various membranes, in which acts as the scavenger of free-oxygen radicals and/or stabilized of the hepatocytes membranes. [Paker, 1991 and Donaldson, 1994]. Also, it could be attributed to increased diniconazole metabolism or excretion and/or activation of anti-oxidative defense systems. Moreover, Yang *et al.* (1992) reported that the selenium supplementation caused statistically significant increment of RNA amount, glutathione peroxidase (GSH-PX) activity and reduction in lipid peroxide in liver rats.

Co-treatment vitamin E or selenium with diniconazole lowered the urea content to the normal level and protected the nephrocytes against oxidative stress. Meanwhile, the results suggest that selenium fall in reducing the level of creatinine to the level of untreated rats. This may be due to the inadequate amount of selenium and/or insufficient the duration of supply. Hypotriglecerdemia was detected in diniconazole-treated rats, but supplemented rats with vitamin E or selenium pre-exposure to diniconazole, led to insignificant differences in the level of TG between antioxidant-supplemented rats and control group. In contrast, hypercholesterolemia was observed in rats following exposure to diniconazole, but supplemented rats with vitamin E or selenium restored the cholesterol levels to the values of control rats.

The low density lipoprotein-cholesterol (LDL-C) acts as a source of cholesterol to peripheral cells, whereas high density lipoprotein-cholesterol (HDL-C) conveys cholesterol from the peripheral cells to the liver [Duncan, *et al.*, 1994]. A significant negative correlation was found between T.chol and HDL-C in diniconazole-treated rats.

Vitamin E or Se supplementation was effective to restore the cholesterol level to the normal values of control rats. These results may be attributed to the ability of Vit. E or selenium in altering the rate of cholesterol or triglyceride metabolism and/or alter the efficiency of absorption the lipids in the intestine of antioxidant-supplemented rats [Sun *et al.*, 1997].

A remarkable reduction in the concentrations of T4 and T3 (Hypothyroidism) was found in the rats post-exposure to diniconazole alone, also the same trend was detected in supplemented rats with tested antioxidants. This could be attributed to inadequate intake of the antioxidants used and/or insufficient the duration of supply. Therefore, the adverse effects of diniconazole in thyrocytes found to be irreversible during the experimental period. Hosokawa *et al.*, (1993) reported that diniconazole induced a significant decrease in the levels of thyroid hormones, which excreted by the liver via increase of hepatic UDP-GT activity. Therefore, the increase excretions of Thyroid hormones cause decrease in serum T4 in circulating blood. In conclusion, all changes seen in haemogram and clinical biochemical markers as well as in the hepatic lipid peroxidation were disappeared in antioxidants-supplemented rats. Wherease, a significant increase in the creatinine level was noted in Vit. E and Se-supplemented rats. However, a decrease in the concentrations of T4 and T3 was continued in the antioxidants-supplemented rats. Consequently, these findings suggest to the protective role of the vitamin E or selenium by ameliorating of the diniconazole-induced cell damage and may be important in enhancing recovery post-exposure to diniconazole.

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الدور الوقائى لفيتامين هـ والسيلينيوم ضد التأثيرات الضارة لمبيد الدينيكونازول الفطرى فى ذكور الجرذان البيضاء

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أجريت هذه الدراسة لتقييم الآثار السامة لمبيد الدينيكونازول الفطرى عند الجرعة ٢٢ مليجرام/كيلو جرام من وزن الحيوان (تمثل ١/١٠ من قيمة الجرعة النصفية المميتة عن طريق الفم لمستحضر الدينيكونازول) فى ذكور الجرزان البيضاء و كذلك الدور الوقائى لفيتامين هـ والسلينيوم كمضادات للأكسدة ضد التأثيرات الضارة للمستحضر الفطرى المختبر.أحدثت المعاملة بالدينيكونازول نقص فى عدد كريات الدم الحمراء وخلايا الدم البيضاء وكذلك سمية كلوية بالاضافة الى سمية كبدية مصحوبة بارتفاع إنزيم الألنين امينوترنس فيريز مصحوبا بأكسدة الليبيدات فى جدر الخلايا الكبدية. وكذلك أحدثت المعاملة زيادة فى مستوى الكوليستيرول مصحوبا بنقص فى الجليسريدات الثلاثية والكوليستيرول العالى الكثافة وكذلك نقص فى هرمونات الغدة الدرقية. ولكن عند اعطاء الحيوانات فيتامين هـ أو السلينيوم قبل المعاملة بالدينيكونازول لوحظ تحسن فى معدل اكسدة الليبيدات وكذلك حدث تحسن فى معايير صورة الدم ووظائف الكبد مصحوبا ذلك بنقص فى معدل أكسدة الليبيدات وكذلك حدث تحسن فى معايير صورة الليبيدات حيث عادت قيمها إلى معدلها الطبيعى بينما لم يحث تحسن فى بعض دلالات وظائف الكلى والغدة الدرقية.

قام بتحكيم هذا البحث: أ.د/زيدان هندى و أ.د/ مصطفى عبدالسميع