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PROTECTIVE EFFECTS OF A COMBINATION OF VITAMINS E AND C PLUS SELENIUM AGAINST THE DINICONAZOLE TOXICITY IN MALE ALBINO RATS

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ABSTRACT

The present study is concerned with the involvement of influence of a combination of vitamins E and C plus Selenium (Se) (at Recommended Daily Allowance, US-RDA) in aemeliorating the toxic effects of diniconazole treatment in male albino rats.

Following oral administration of diniconazole to rats in dosage of 22mg/kg B.Wt, (at dose represent 1/10 of oral LD_{50}) there was haematological changes, and hepatic lipid peroxidation, along with elevating of serum ALT activity (alanine aminotransferase) and an inhibition of AST (aspartate aminotransferase) and ALP activity (alkaline phosphatase), as well as a marked disturbance in the lipid metabolism of diniconazole-treated rats. Also, there was impairs in the kidney and thyroid function. But when supplemented rats with a combination of vitamins E and C plus selenium and then treated with diniconazole an improving in haematological findings was observed and biochemical alterations of liver enzymes and kidney function are missing. Also, the hepatic lipid peroxidation was suppressed, in addition to lowered serum total and low density lipoprotein – cholesterol (TC & LDL-C) concentrations, with elevating level of hight density lipoprotein – Cholesterol (HDL–C), however, this combination succeed to improve the thyroid hormones metabolism especially, T_3 (Triiodothyronine) production.

Key words: Vitamin E – Vitamin C – Selenium – Diniconazole – Cholesterol.

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 respectively for 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) Weishelbaum (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 Commercial 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 (MDA) is measured in the spectrophotometer at 532 nm.

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

The results presented in Table (1) demonstrated that no-significant changes in erythrocyte count was observed in rats treated with diniconazole alone or with a combination of vitamins E and C plus Selenium (Se) element. Meanwhile, a significant elevation in the haemoglobin concentration (Hb) and mean corpuscular haemoglobin concentration (MCHC) was observed in diniconazole – treated rats after 7 and 21 days of treatment. Also, this trend was noticed in rats supplemented with combination of vitamins E and C plus Se pre-treatment with diniconazole for 7 days of treatment.

Also, there was a remarkable elevation in the packed cell volume (PCV) in diniconazole – treated rats after 21 and 28 days of treatment, but when supplemented rats with a combination of vitamins E and C plus Se, pre-exposure to diniconazole, restored the PCV values to the normal level of control group.

In contrast, a significant decrease in the total leukocyte count (WBCs) as well as in the neutrophils and lymphocytes leukocytic counts was observed in rats following treatment with diniconazole.

Administration rats with combination of vitamins E and C plus Se, pre-treatment with diniconazole, returned the WBCs count as well as the neutrophils and lymphocytes count to the normal limits of control group.

As shown in Table (2), there was a marked increase in the activity of plasma alanine aminotransferase (ALT), whereas a significant decline in the activity of aspartate aminotransferase (AST) was found in rats post-exposure to diniconazole after 28 days. Supplementation rats with combination of the three tested antioxidants, prior to treatment with diniconazole resulted in a significant increase in the activity of AST after 7 and 14 days, but this elevation returned to normal level in the rest of the experimental period.

However, in the present study, there was a tendency toward adecline markedly in the activity of alkaline phosphatese (ALP), in rats treated only with diniconazole within the experimented time, but

Table (1) : Changes in haematological parameters from rats orally exposed to diniconazole alone or with a combination of vitamins E and C plus Se as a protective agents for 28 days.

Time	7 days			14 days			21 days			28 days		
Treatment / Parameter	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din
RBCs $X10^6/\mu l$	6.305 ± 0.308	7.128 ± 0.240	7.412 ± 0.54	6.798 ± 0.310	7.574 ± 0.513	6.435 ± 0.342	6.478 ± 0.407	6.462 ± 0.152	6.252 ± 0.675	5.798 ± 0.488	5.550 ± 0.394	5.320 ± 0.218
Hb g/dl	9.75 ± 0.55	12.48 ± 0.429**	12.42 ± 0.566**	9.346 ± 0.624	10.877 ± 1.110	10.891 ± 0.643	10.010 ± 0.296	12.676 ± 0.603	10.807 ± 0.559	10.108 ± 0.480	9.041 ± 1.046	8.909 ± 0.548
PCV %	46.00 ± 2.07	50.40 ± 2.014	53.40 ± 2.13*	45.40 ± 2.94	48.40 ± 3.218	50.40 ± 2.767	42.40 ± 1.568	50.40 ± 1.60**	47.00 ± 2.664	44.98 ± 0.67	49.23 ± 0.52**	46.200 ± 4.079
MCV ft	74.88 ± 4.941	69.685 ± 2.305	73.08 8 ± 4.172	76.52 ± 5.04	64.761 ± 6.150	74.994 ± 4.472	73.005 ± 5.36	78.049 ± 2.194	69.250 ± 3.56	76.55 ± 5.08	79.018 ± 6.951	76.917 ± 3.586
MCH Pg	14.97 ± 1.71	18.258 ± 0.697	16.985 ± 0.960	15.65 ± 1.67	14.567 ± 1.836	16.082 ± 0.365	14.786 ± 1.766	17.21 ± 0.850	18.502 ± 2.386	15.57 ± 1.66	16.423 ± 1.949	17.451 ± 0.958
MCHC %	20.81 ± 0.942	22.128 ± 1.16	23.423 ± 1.384*	20.414 ± 1.362	22.274 ± 1.202	21.821 ± 1.640	21.420 ± 0.79	25.103 ± 0.419**	21.897 ± 1.989	18.871 ± 1.569	20.674 ± 1.179	19.716 ± 1.467
WBCs $X10^3/\mu l$	11.470 ± 0.33	9.470 ± 0.857	19.187 ± 0.81***	11.650 ± 0.274	10.610 ± 0.99	11.33 ± 1.157	11.930 ± 0.136	10.19 ± 0.808	9.440 ± 1.740	11.940 ± 1.36	7.083 ± 0.346**	11.931 ± 2.526
Lymphocytes $X10^3/\mu l$	9.069 ± 0.879	8.015 ± 0.089	11.980 ± 1.338	9.952 ± 0.254	9.234 ± 0.812	11.717 ± 0.669	9.269 ± 0.432	7.96 ± 0.47	10.490 ± 1.154	9.380 ± 1.382	6.128 ± 0.283*	10.229 ± 0.938
Neutrophils $X10^3/\mu l$	1.770 ± 0.208	1.409 ± 0.075	1.512 ± 0.135	1.647 ± 0.287	1.660 ± 0.723	1.374 ± 0.115	1.493 ± 0.254	1.202 ± 0.051	1.012 ± 0.103	1.381 ± 0.044	0.915 ± 0.117*	1.136 ± 0.194

Data represented in Mean ± S.E.M. for 5 rats
Din = Diniconazole Fungicide comb.= combination of three antioxidants (vitamins E, C and Se) RBCs = Red Blood Cells Hb= Haemoglobin
PCV = Packed Cell Volume MCV= Mean Corpurcular Volume MCH = Mean Corpuscular haemoglobin
MCHC= Mean Corpuscular haemoglobin Concentration. WBCs = White Blood Cells
* Statistically significant in comparison with the control group ($p<0.05$) ** Statistically significant in comparison with the control group ($p<0.01$)
*** Statistically significant in comparison with the control group ($p<0.001$)

Table (2) : Changes in serum liver enzymes and proteins concentrations from rats orally exposed to diniconazole alone or with a combination of vitamins E and C plus Se as a protective agents for 28 days

Time	7 days			14 days			21 days			28 days		
Treatment / Parameter	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din
ALT U/L	37.940 ± 5.10	36.91 ± 5.70	53.296 ± 3.828*	32.310 ± 6.090	50.65 ± 5.70	39.629 ± 3.61	33.254 ± 5.435	50.51 ± 4.6*	38.515 ± 3.168	37.20 ± 0.210	50.647 ± 3.98*	34.027 ± 4.725
AST U/L	102.082 ± 5.910	89.166 ± 5.796	137.491 ± 5.477***	96.288 ± 7.109	108.11 ± 7.75	128.606 ± 9.945*	94.542 ± 5.068	110.416 ± 7.963	97.638 ± 6.862	105.50 ± 7.70	79.721 ± 8.019*	120.971 ± 11.341
ALP U/L	27.614 ± 5.465	36.329 ± 2.935	30.090 ± 4.037	31.237 ± 2.427	10.916 ± 1.621***	28.797 ± 1.816	29.128 ± 2.397	10.549 ± 2.620***	33.806 ± 4.364	29.311 ± 2.940	22.339 ± 0.530*	26.833 ± 3.080
S.T.P g/dl	6.462 ± 0.269	6.356 ± 0.435	6.462 ± 0.269	6.205 ± 0.432	5.362 ± 0.539	5.780 ± 0.307	5.852 ± 0.349	5.498 ± 0.158	6.843 ± 0.628	5.085 ± 0.276	7.931 ± 0.380**	5.951 ± 0.500
Alb. g/dl	3.722 ± 0.431	2.678 ± 0.438	3.722 ± 0.431	4.221 ± 0.221	2.880 ± 0.620	4.572 ± 0.166	3.987 ± 0.300	3.938 ± 0.150	4.525 ± 0.179	3.586 ± 0.294	2.847 ± 0.267	4.240 ± 0.528
glob. g/dl	2.842 ± 0.670	3.678 ± 0.256	2.842 ± 0.670	2.415 ± 0.357	2.58 ± 0.62	2.112 ± 0.162	2.082 ± 0.246	2.938 ± 0.842	2.318 ± 0.166	2.544 ± 0.471	5.074 ± 0.272***	2.164 ± 0.370

ALT = Alanine aminotransferase AST = Aspartate aminotransferade ALP = Alkaline Phosphatase

S.T.P = Serum Total Protein Alb.= Albumin glob.= globulin

* Statistically significant in comparison with the control group ($p<0.05$) ** Statistically significant in comparison with the control group ($p<0.01$)

*** Statistically significant in comparison with the control group ($p<0.001$)

receiving rats a combination of vitamins E and C plus Se, restored the activity of ALP to the normal level of untreated rats.

Meanwhile, a significant elevation in the serum total protein (S.T.P) and globulins level was observed in rats following exposure to diniconazole. However, supplemented rats with a combination of two vitamins (i.e., E & C) and Se, pre-treatment with diniconazole, restored the level of T.P and globulins to the normal level of control rats (Table 2)

Malondialdehyde (MDA) level in whole hepatic homogenate of diniconazole – treated rats was significantly higher than of the control group at the end of experiment (28 days). But when supplemented rats with a combination of vitamins E and C plus Se with diniconazole, a marked decrease in MDA was noticed in compared with control group (Table, 3).

Furthermore, there was a significant elevation in the urea levels in rats, post-exposure to diniconazole alone after 7, 21 and 28 days of exposure, as well as this trend was observed in the creatinine level in diniconazole – treated rats after 28 days of treatment. Supplementation rats with a combination of vitamins E and C plus Se, pre-treatment with diniconazole, resulted in returned the values of these parameter to the normal level of untreated rats. (Table, 4)

A significant reduction in the Triglycerides (TG) levels was noticed in rats following exposure to diniconazole throughout the experimental period. Meanwhile, a significant increase in the levels of total cholesterol (TC) and low densitylipoprotein-cholesterol (LDL-C) was recorded in diniconazole – treated rats after 21 days and 28 days of treatment. Contrary to this, a significant reduction in the levels of high density lipoprotein- cholesterol (HDL-C) was observed in rats treated only with diniconazole throughout the experimental period. (Table, 5)

Supplementation rats with a combination of vitamins E and C plus Se, with diniconazole, restored the levels lipid profile parameter (i.e., TG, TC, LDL-C and HDL-C) to the normal level of control rats

A remarkable reduction in the concentrations of Thyroxine (T_4) and Triiodothyronine (T_3) was recorded in diniconazole – treated rats, but when supplemented rats with the mixture of the three antioxidants (i.e., E, C and Se), pre-treated with diniconazole, restored only the levels of T_3 to the normal level of control group, whereas a marked

Table (3) : Changes in hepatic lipid peroxidation (MDA) from rats orally exposed to diniconazole alone or with a combination of vitamins E and C plus Se as protective agents or 28 days

Parameter	Cont.	%	Din.	%	Comb.+Din	%
MDA nmol/g of wet liver tissue	355.38 ± 8.875	100 %	411.90 ± 9.36**	115.902%	221.89 ± 22.33**	62.44%

MDA = Malondialdehyde

** Statistically significant in comparison with the control group ($p<0.01$)

Table (4) : Changes in kidney function parameter from rats orally exposed to diniconazole alone or with acombination of vitamins E and C plus Se as a protective agents for 28 days

Time	7 days			14 days			21 days			28 days		
Treatment / Parameter	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din
Urea mg/dl	16.876 ± 1.426	26.440 ± 0.97***	17.09 ± 1.27	18.411 ± 1.595	16.460 ± 0.860	16.110 ± 0.92	17.883 ± 1.121	22.748 ± 0.842**	22.69 ± 2.370	17.677 ± 1.910	24.11 ± 1.340**	16.933 ± 1.671
Creatinine mg/dl	6.100 ± 0.509	5.172 ± 0.230	5.16 ± 0.240	5.70 ± 0.460	5.829 ± 0.570	6.00 ± 0.150	5.800 ± 0.300	9.00 ± 0.200**	6.66 ± 0.590	6.050 ± 0.460	11.35 ± 0.580***	6.613 ± 0.573

** Statistically significant in comparison with the control group ($p<0.01$)

*** Statistically significant in comparison with the control group ($p<0.001$)

Table (5) :Changes in lipid profile from rats orally exposed to diniconazole alone or with acombination of vitamins E and C plus Se as a protective agents for 28 days

Time	7 days			14 days			21 days			28 days		
Treatment / Parameter	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din	Cont.	Din.	Comb.+Din
TG. mg/dl	39.209 ± 1.977	24.189 ± 3.917**	35.172 ± 4.571	37.657 ± 3.617	29.406 ± 2.590*	89.011 ± 0.851***	37.66 ± 1.77	28.30 ± 1.702*	71.304 ± 4.259***	36.448 ± 1.288	18.339 ± 1.940**	39.525 ± 5.994
TC mg/dl	48.768 ± 4.60	51.87 ± 5.58	59.609 ± 5.667	48.648 ± 3.295	55.104 ± 40.070	45.945 ± 1.816	48.167 ± 4.715	60.360 ± 0.300**	43.684 ± 2.066	44.636 ± 3.477	61.411 ± 3.280***	45.044 ± 0.349
L.D.L.-C mg/dl	33.49 ± 2.57	28.25 ± 1.40	34.996 ± 1.993	32.04 ± 1.66	46.419 ± 13.20	35.840 ± 1.404	34.680 ± 3.030	58.170 ± 0.80**	31.20 ± 3.69	33.540 ± 0.432	67.040 ± 3.600**	37.947 ± 4.624
H.D.L.-C mg/dl	21.472 ± 2.028	13.78 ± 0.654***	20.737 ± 1.416	21.682 ± 1.952	16.45 ± 0.313*	23.310 ± 0.518	22.855 ± 1.241	15.197 ± 1.630**	18.427 ± 1.561	21.273 ± 1.334	14.560 ± 0.432*	20.475 ± 1.875

TG = Triglycerides TC = Total Cholesterol L.D.L–C = Low density Lipoprotein – Cholesterol

HDL–C = High density Lipoprotein-Cholesterol

* Statistically significant in comparison with the control group ($p<0.05$) ** Statistically significant in comparison with the control group ($p<0.01$)

*** Statistically significant in comparison with the control group ($p<0.001$)

reduction in the concentration of T_4. persisted in antioxidants–supplemented rats (Table, 6).

Table (6) : Changes in thyroid hormones (T_4 & T_3) from rats orally exposed to diniconazole alone or with a combination of vitamins E and C plus Se as protective agents for 28 days

Parameter	Cont.	Din	Comb.+Din
T_4 μ g/dl	48.72 ± 6.27	30.260 ± 1.709*	28.101 ± 1.957***
T_3 ng/dl	1.920 ± 0.191	1.049 ± 0.092**	1.959 ± 0.200

T_4 = Thyroxine T_3 = Triiodothyronine
* Statistically significant in comparison with the control group ($p<0.05$)
** Statistically significant in comparison with the control group ($p<0.01$)
*** Statistically significant in comparison with the control group ($p<0.001$)

DISCUSSION

Reactive oxygen species [ROS] are essential for life of aerobic organisms. They produced in normal cells and formed as a result of exposure to numerous factors both chemical and physical.

In normal cells, active oxygen derivatives are neutralized or eliminated owing to the presence of a natural defense mechanism. These defenses mechanism involve, enzymatic antioxidants and water or fats soluble non-enzymatic antioxidants (vitamins C, E, glutathione and Selenium). However, the capacity of the organism for enhancement of antioxidant defenses, will depend in part upon the previous and concomitant intake of nutrients (Gaby *et al.*, 1996).

In addition, the antioxidant functions of vitamin C and E are being widely investigated because they may be a connection between the amounts of these micronutrient consumed and the amount of oxidative damage leading to cancers and degenerative diseases. [Ames, 1983]. Our results indicated the occurance of leucopenia associated with neutropenia and lymphopenia, which occurred as a responsive to diniconnazole treatment, this may be due to increased destruction and/or increased sequestration of lymphocyte in lymphoid or other tissue because of altered surface properties. (Wong *et al.*, 1992 and Tvedten, 1994). The results obtained from the experiment

showed that supplementation rats with a combination of vitamins E and C plus Se, pre-exposure to diniconazole, improved the haematological findings, [i.e., leucopenia, neutropenia and lymphopenia] which induced by dinconazole alone. This may be occurred as a result of stimulation of myelopioesis in bone marrow of supplemented rats. These appears to be appreciable evidence that vitamin C is involved in immune response through participating in the maintenance of phagocytesis. Also, it reduces allergic reactions and enhancement of leukocyte function [Anderson *et al.*, 1980 and Anderson, 1981].

However, Baraboi and Shestakova (2004) found that selenium is active immunomodulator much more patient antioxidants than vitamin E, C and B- carotene. Also, selenium affects all components of the immune system and is necessary for its optimum performance [Sies and Stahl, 1995].

Our results in previous study (Shama *et al.*, 2006) suggest that supplementation rats with vitamin E and selenium (Se) individually, pre-treatment with diniconazole improving haematopioesis (i.e., erythropioesis and myelopioesis). Also, Eman (2002) found that supplementation rats with vitamin C individually at 200mg/day pre-treatment with diniconazole produced a considerable improvement in haematological parameters impaired by diniconazole.

However, our results suggest that diniconazole treatment increased the activity of ALT and the hepatic lipid peroxdation. This may be due to a decrease of antioxidant status and consequently increasing of oxidative stress in hepatic tissue [Acworth *et al.*, 1997). But administration rats of a combination of selected vitamins (i.e., E & C) and selenium, prior to exposure to diniconazole, considerably reduce the hepatotoxicity, which reflected by decreasing the MDA level and returned the activity of ALT and AST to the normal level. Meanwhile, a decline in the activity of ALP was noticed in rats following treatment with diniconazole, this may be attributed to diniconazole toxicity involves the binding of magnesium ions, and consequently inhibition of Mg-dependant enzymes, such as alkaline phosphatase and/or hypothyrodism, which induced by diniconazole as shown in our results. [Mysliwiec, 2002] . Concomitant use of a combination of vitamins E and C plus Se, with diniconazole, succeed to restore the activity of ALP to normal level of control group.

It has reported that supplementation rats with vitamin E or Se alone, pre-treatment with diniconazole, has prononunced a protective effect by combating lipid peroxidation in hepatocytes and this concomitant with diminishing the activities of ALT, AST and ALP and consequently the hepatotoxicity or oxidative stress in hepatocytes (Shama *et al.*, 2006).

Also, oral administration rats with vitamin C, pre-exposure to diniconazole, restored the activities of liver enzymes viz. ALT, AST and ALP and also the hepatic lipid peroxidation level, approximately to the normal level of un-treated rats (Eman, 2002).

However, Negre-Salvayre *et al.* (1995) found that the mixture of the three compounds (rutin/ascorbic acid/$\propto$-Tocopherol, 4/4/1) exhibited a supra-additive antioxidant effect. The antioxidant mixture permitted a maximal cytoprotective effect with relatively lower concentration.

The hyperproteinemia was observed as a consequence of hyperglobulonemia in rats treated only with diniconazole. This occurred could be as a result of polyclonal hyperglobulinemia, also referred to gammapathies have abroad - based peak in the beta and gamma regions and suggest chronic inflammatory [Roth, 1995].

Simultaneous administration of the mixture of the three antioxidants (i.e., vitamins E and C plus Se), reduced the hyperproteinemia and hyperglobulinemia to the normal limits of control group. Rats had hypotriglycerdemia and hypercholesterol-emia, accompanied by elevating of LDL-Cholesterol following treatment with diniconazole. This could be attributed to deliverting the hepatic in flow of free fatty acids from the pathways of estrification into these oxidation, and thus decreasing the secretion of TG by the liver to the circulating blood. [Mayes, 1993]. Several investigators showed that a block of the secretion of hepatic TG into plasma, is the major mechanism underlying the fatty liver induced in rats. [Zilva *et al.*, 1988 and Stein, 1996].

The hypercholesterolemia in diniconazole-treated rats was occurred and this may be attributed to a reduction in LDL-receptors in the liver, as a result of hypothyrodism and thus retarding LDL-C catabolism and/or to a decrease of HDL-C level as shown in our results. This, in turn, may be due to impairment in biosynthesis of HDL-C as a secondary effect of liver damage [Teitz, 1989].

However, the present results suggest that the combination of two vitamins E and C plus Se, has most effective in lipid metabolism, where the antioxidants supply sought to restore the levels of TC and LDL-C approximately to the level of control group and thus minimize the risk of coronary heart disease (CHD) and this risk falls as the level of HDL-C increases.

A previous study (Shama *et al.*, 2006) demonstrated that supplementation rats with vitamin E and Se, separately with diniconazole produced a marked decrease in the levels of total cholesterol and more harmful LDL-Cholesterol level, concomitantly with enhancing of HDL-Cholesterol level (good cholesterol) to the normal level of control group.

Tawfek and Taha (2006) found that diabetic rats supplemented with either antioxidants (Taurine and Selenium) or immuno-suppressive agent (azathioprine) showed a decrease in serum Triglycerides and LDL-Cholesterol level and raised of serum HDL-Cholesterol level significantly when compared with diabetic rats.

Likewise, vitamin C, when given to rats at 200mg/day, pre-treatment with diniconazole, exhibited a hypo-cholesterolaemic action and a significant increase in HDL-Cholesterol approximately to the normal level was observed (Eman, 2002).

In 1984, Holloway *et al.*, found that vitamin C stimulates the activity of cholesterol. 7-alpha hydroxylase, which regulating the conversion of cholesterol to bile acids, since bile acids are the vechile for cholesterol excretion. Therefore, vitamin C might, in turn, lower blood cholesterol concentrations. However, the reports are controversial, which declared that vitamin C intake does not consistently correlate with total serum cholesterol in humans (Lapidus *et al.*, 1986). The beneficial effect of vitamin C may be through an increase in HDL-C, the so-called "good cholesterol" rather than through a lowering of total cholesterol (TC). Also, it has been suggested that vitamin C significantly alters cholesterol levels only in hypercholesterolemia subjects [Ginter *et al.*, 1977] .

Also, an elevation markedly in the levels of urea and creatinine was observed in dinicnazole-treated rats. This may be due to dehydration of diniconazole treated rats, which in turn, can cause hypovolemia, that lead to impaired excretions of urea or creatinine, secondary to reduced renal blood flow (RBF) and glomerular

filtration (GFR). [Finco, 1989 and Lees *et al.*, 1994]. But administration rats of combination of the three antioxidants, (i.e., E, C & Se) improving or increasing excretory function of kidney. Therefore, the combination of vitamins E and C plus Se succeed to lessened the impairment of kidney function caused by diniconazole.

Rats were received vitamin E and Se individually before treatment with diniconazole, exhibited improve of renal function (Shama *et al.*, 2006). In contrast, supplemented rats with vitamin C concomitant with diniconazole did not produce any improvement in excretory function of kidney (Eman, 2002).

Hosoakawa *et al.* (1993) found that diniconazole treatment increased markedly of excreting thyroid hormones via the liver by enhancing of hepatic UDP-GT activity, this in turn, lead to decrease of the serum T_4 and T_3.

The hypothyrodism in rats was occurred as a result of diniconazole treatment. Meanwhile, supplementation rats with such combination, restored the T3 concentration of the normal level to the control group.

In contrast, supplementation rats with vitamins E and C and Se individually, pre-exposure to diniconazole, fail to overcome on the impairment of production of T_4 and T_3.

Beckett and Arthur (2005) reported that the trace element selenium (Se) is modifying the expression of at least 30 selenproteins well -characetrized selenoenzymes, such as glutathione peroxidase (GPXs) and iodothyronine deiodenase (Ds), which are capable of modifying thyroidal Dl in rats and both Dl and D2 in humans are also up-regulated to increase the production of bioactive 3, 5\3 triiodothyronine (T3). In the basal state, may down - regulate thyroid hormones.

From these results, it can be concluded that *in vivo*, administration of diniconazole to male albino rats resulted in hematological changes and induction of hepatic lipid peroxidation along with elevating of serum ALT activity, and a decrease of ALP activity, in addition to a significant alteration in lipid profile and impairment of kidney function as well as hypothyroidism induced by diniconazole was observed. These results suggest that reactive oxygen species (ROS) or free radicals may be involved in the toxic effects of diniconazole. However, a combination of vitamins E and C plus Se permitted cytoprotective effect reflected on improving the

haemogram, suppression of hepatic lipid peroxidation and reduce the nephrotoxicity as well as improved the lipid and thyroid gland metabolism.

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التأثيرات الوقائية لخليط من فيتامينات هـ وسي والسيلنيوم ضد سمية الدينيكونازول في ذكور الجرذان البيضاء

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باحث أول – قسم سمية المبيدات للثدييات والأحياء المائية – المعمل المركزي للمبيدات – مركز البحوث الزراعية الدقى – الجيزة

تتناولت هذه الدراسة تأثير خليط من فيتامينات هـ وسي والسيلنيوم (عند الجرعات المسموح بها) في تحسين الأثار السامة لمبيد الدينكونازول في ذكور الجرذان البيضاء حيث أحدث إعطاء الجرذان لمبيد الدينيكونازول عن طريق الفم بجرعة ٢٢ مليجرام/كيلوجرام من وزن الجسم (تمثل ١٠/١ من الجرعة القاتلة النصفية عن طريق الفم لذكور الجرذان البيضاء) تغيرات هيماتولوجية كما أحدث أكسدة للبيدات في أنسجة الكبد مع ارتفاع في نشاط أنزيم الألينين ترنسفيريز وهذا يشير إلى حدوث سمية كبدية كما حدث تثبيط لأنزيم الفوسفاتيز القاعدي واضطراب معنوي في تمثيل الليبيدات في الجرذان المعاملة بالدينيكونازول. من ناحية أخرى حدث خلل في وظيفة الكلى ونشاط الغدة الدرقية ولكن عند إعطاء هذه الجرذان خليط من فيتامينات هـ وسي والسيلينيوم قبل معاملتها بالدينكونازول لوحظ تحسن في التغيرات الهيماتولوجية وكذلك اختفت الشواهد البيوكيميائية الخاصة بوظائف الكبد والكلى كما حدث تثبيط لأكسدة الليبيدات في أنسجة الكبد. علاوة على ذلك قد أحدث هذا الخليط حفظ لمستوى الكوليستيرول الكلي والليبوبروتينات منخفضة الكثافة وذلك مع ارتفاع مستوى الليبوبروتينات عالية الكثافة وذلك لمستوى المجموعة الضابطة وهذا الاتجاه لوحظ مع الجليسريدات الثلاثية. من ناحية أخرى نجح هذا الخليط في تحسين وظائف الغدة الدرقية وخاصة إنتاج التراي ايدوثيرونين.