Study of some hazard of difenoconazole on rats with trials to overcome its deleterious effects

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Seventy-five rats were used to determine the toxic effects of difenoconazole (D) fungicide on haematological, some biochemical parameters and thyroid hormones in adult albino rats of both sexes, as well as possible inhibition of adverse effects by using vitamin E and/or vitamin C as antioxidant drugs.

Rats were classified into five groups each of 15 rats. Group (1) served without treatment as negative control, group (2) treated with 1/10 of the LD_{50} of D (140.3 mg/kg B.wt.), in alternative days for 30 days and cessation of D for further 15 days as a withdrawal period, group (3) treated with vit. E (24 mg/ kg B.wt.) combined with the same dose of D, intubation every other day, group (4) administered vit. C (200 mg/ kg B.wt.) daily per os combined with the same dose of D in alternative days, group (5) taken vit. E, vit. C and D. Vitamins were given daily from the beginning of experiment till the end and at withdrawal period. Samples were collected at 15 and 30 days during D exposure and at the end of withdrawal period.

Using analysis of variance, R.B.Cs. count, PCV % and Hb content significantly decreased than negative controls allover the experimental period in D- treated groups, however vit. C alone or combined with vit. E produced gradual improvement in haematological parameters. Marked elevation in W.B.Cs. count and lymphocytes, accompanied by neutropenia were observed in all groups compared to negative controls allover the experiment period.

Total protein, triglyceride and high-density lipoprotein levels were significantly reduced in all D-administered groups compared to negative controls. Aspartate aminotransferase, alanine aminotransferase, urea, creatinine, cholesterol and low density lipoprotein showed higher levels in all D-treated groups in comparison to negative controls, meanwhile, administration of vit. E and/or vit. C produces gradual improvement in these parameters.

Thyroid hormonal analysis indicated significant decrease in triiodothyronine (T_3) and thyroxine (T_4) in all D- treated groups, whereas their levels returned to normal in groups given vit. C alone or combined with vit. E at the withdrawal period.

In conclusion, these results indicated that D fungicide exposure caused overt disorders in haematological and biochemical parameters as well as thyroid function. These effects could be ameliorated by using vit. E and vit. C as antioxidant drugs. Vit. E and vit. C when combined together produce best results in all tested parameters, however vit. C alone has rapid response than vit. E especially in haematological picture and thyroid function.

Difenoconazole is a triazole fungicide that exhibits its antifungal activity by inhibiting fungal ergosterol biosynthesis. Triazoles fungicides are economically important agricultural chemicals as they are widely used on crops such as wheat, barley and orchard fruits (Nikolay *et al.*, 2001).

Triazoles are both curative and preventative with longer residual properties than most fungicide chemistries (Schuh and Butzen, 2004). Free radicals are formed primarily in the body during normal metabolism and also upon exposure to environmental factors such as cigarette smoke or other pollutants. Fat an integral part of all cell membrane vulnerable to destruction through oxidation by free radicals (Traber and Packer, 1995). Free radicals can damage cells and may contribute to the development of cardiovascular disease and cancer (Farrell and Roberts, 1994).

The fat-soluble vitamin, α-tocopherol, is uniquely suited to intercepting free radical preventing a chain reaction of lipid destruction. Aside form maintaining the integrity of cell membranes throughout the body; α-tocopherol also protects fat low-density lipoproteins (LDLs) from oxidation. Lipoproteins are particles composed of lipids and proteins, which are able

to transport cholesterol from the liver to the tissues of the body. Oxidized LDLs have been implicated in the development of cardiovascular diseases. When a molecule of α-tocopherol neutralizes a free radical, it is altered in such a way that its antioxidant capacity is lost. However, other antioxidants, such as vit. C, are capable of regenerating the antioxidant capacity of α-tocopherol (Traber, 1999).

Vit. C, known as ascorbic acid, is a water soluble vitamin. It is a highly effective antioxidant. Even in small amounts, vit. C can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants. It acts as direct scavenger of free radicals (Carr and Frei, 1999).

Due to great economic losses from the toxicity by pesticides, the present work was done in order to assess the adverse effects of difenoconazole and whether vit. E and/or vit. C have protective effects against its toxicity.

Material and Methods

Drugs.

Difenoconazole (D). It is a triazole fungicide with chemical name, cis, trans- 3- chloro- 4- [- 4- methyl-2- (1H- 1, 2, 4- triazole- 1- methyl)- 1, 3- dioxylpentane 2-] phenyl- chlorophenyl ether, and molecular formula $C_{19}H_{17}Cl_2N_3O_3$. It is yellowish white crystals mixed easily with water. It was obtained from CIBA Geigy Company.

Vitamin E (Alpha-tocopherol). It is a fat-soluble vitamin, purity 98- 100% with chemical structure $C_{29}H_{50}O_2$. It was obtained from Merck, Co., Germany in oily liquid preparation.

Vitamin C (Ascorbic acid). It is a water-soluble vitamin, purity 99.9% with chemical structure $C_6H_8O_4$. It was obtained in a powder form, from Merck Co., Germany.

Experimental animals. Seventy-five adult Wister albino rats of both sexes weighing from 120 to 150g were used.

Experimental design. Rats were divided into five equal groups (15 of each) as follows: Group 1: was kept without treatment as negative control.

Group 2: was orally treated with D for 30 days every other day (140.3 mg/ kg B.wt.) equivalent to 1/10 LD_{50} (Pesticide Manual, 1994). Group 3: was administered daily oral dose of vit. E (24 mg/kg B.wt) according to Paget and Barnes (1964), 4 hours before D administration. Group 4: given vit. C (200 mg/kg. B.wt) daily according to Paget and Barnes (1964) per os, 4 hours before D administration. Group 5: given vit. E, vit. C and D.

The period of experiment was extended to 45 days. D was given for 30 days in alternative days in all groups and stopped for the following 15 days as a withdrawal period. However, vitamins were given daily from the beginning of experiment till the end, and also after cessation of D exposure during the withdrawal period.

Sampling. Five rats from each group were sacrificed at 15, 30 days after D exposure and at the end of withdrawal period (45 days). Blood samples were collected from cervical vein (Halperin *et al.*, 1951) and divided into 2 portions, one mixed with EDTA for haematological examination, while serum was separated from the second portion for biochemical and thyroid hormones analysis.

Haematological examination. Erythrocyte count, haematocrite value, haemoglobin concentration, erythrocytic indices, white blood cells and differential leucocytic count were determined according to Schalm (1986).

Biochemical analysis. Serum parameters were determined colourimetrically using diagnostic reagents (Bio-Merieux kits, France). Determination of total protein was performed according to (Sonnenwirth and Jarette (1980), aminotransferases enzymes (Reitman and Frankel, 1957), urea (Patton and Crouch, 1977), creatinine (Husdan and Rapaport, 1968), triglycerides (Fossati and Prencioe, 1982), total cholesterol (Stein, 1996), low and high density lipoproteins (Pesce and Kaplan, 1987).

Hormonal assay. Estimation of serum total triiodothyronine (T_3) and total thyroxine (total T4) hormones was performed as described by Britton *et al.* (1975); using Diagnostic Product Corporation (DPC) kits.

Statistical analysis. The obtained data were statistically computerized using analysis of variance (ANOVA) and least significant difference (LSD) at $P < 0.05$ using SPSS 11 (2002).

Results

Data displayed in (Table 1) demonstrates the blood picture of experimental groups during and after difenoconazole (D) exposure and also during the withdrawal period.

At 15 days of the experiment groups 2-5 showed marked reduction ($P < 0.05$) in R.B.Cs., PCV% and Hb values, whereas erythrocytic indices did

not significantly differ than negative control. After 30 days of D administration, results concerning groups 2 and 3 recorded a depression values (P< 0.05) in R.B.Cs., PCV% and Hb content, while groups 4 and 5 showed no significant difference in comparison to negative controls. However, no differences in erythrocyte indices were found in all experimental groups.

At the withdrawal period, all haematological values returned to normal levels in groups 3-5. However, the group treated with D only showed reduction in R.B.Cs., PCV% and Hb content at (P< 0.05) compared to –ve control.

Results in (Table 2) indicated marked increase in number of W.B.Cs. (P< 0.05) and lymphocytes % (P< 0.05), however, neutrophils % was significantly decreased (P< 0.05) in all D-treated groups with or without antioxidants from day 15 till the end of experiment.

The effects of D administration alone or with oral intubation of vit. E and/or vit. C on some biochemical parameters all over the experimental and withdrawal periods were shown in (Table 3).

At 15 days of D-exposure total protein (T.P) was significantly decreased (P< 0.05) in groups 2-5 compared to negative controls.

At the 30th day of experiment groups 2-4 showed depression in T.P value (P< 0.05) in comparison to –ve controls, while group 5 recorded normal T.P value.

At withdrawal period, groups intoxicated with D and given antioxidant drugs, reported improved T.P values, meanwhile group 2 administered D alone showed a significant decrease (P< 0.05) in T.P level compared to negative controls.

Results in (Table 3) showed high activity (P< 0.05) of ALT, AST, urea and creatinine levels in all groups at day 15 of experiment, meanwhile at the 30th day, all groups showed significant (P< 0.05) rise in ALT and creatinine levels, while G 5 only showed no variation compared to negative controls in AST and urea levels. At withdrawal period groups 3-5 recorded normal ALT and AST values, however urea and creatinine returned to normal values in group 5 only.

Effect of D exposure alone or with vit. E and/or vit. C on cholesterol (Ch), triglycerides (TG), low and high-density lipoprotein (LDL) and (HDL) were shown in (Table 4). At the 15th day of work, group 2 showed significant increase in Ch and LDL (P< 0.05) with reduction in TG and HDL (P< 0.05) levels in comparison to negative controls. Level of Ch, still significantly elevated (P< 0.05) in all other groups, however groups 3-5 showed rapid responses in TG, LDL and HDL values. After 30 days of D exposure, Ch recorded higher levels (P< 0.05) in all groups, whereas group 2 showed increase in LDL (P< 0.05) with depression (P< 0.05) in TG and HDL values, meanwhile groups 3-5 reported no significant variations in TG, LDL and HDL values compared to negative controls. At the withdrawal period, all biochemical parameter values returned to normal levels in groups administered D with vit. E and/ or vit. C. However, those received D alone showed a significant increase (P< 0.05) in Ch and LDL with a significant (P< 0.05) reduction in TG and HDL values compared to negative controls.

Table (5) represented the effect of D alone or with vit. E and/or vit. C on thyroid hormones. Marked reduction in Triiodothyronine (T_3) and Thyroxin (T_4) levels in all groups at the 15th and the 30th days of experiment was recorded, meanwhile at withdrawal period, groups 4 and 5 showed no significant changes in T_3 and T_4 levels compared to negative control. However, groups 2 and 3 showed significantly decreased values (P< 0.05) in comparison to negative controls.

Discussion

The wide spread use of fungicides is usually connected with problems of pollution and health hazards. Difenoconazole (D) is the active ingredient of a fungicide that offers broad-spectrum control of several seeds, soil borne, and foliar pathogens of wheat (EPA, 2001).

Antioxidants such as vit. E and vit. C act to protect cells against the effects of free radicals, which are potentially damaging by-products of energy metabolism (Traber, 1999).

In the current work, haematological, biochemical and thyroid hormones were studied in adult albino rats of both sexes administered orally with $^1/_{10}$ LD_{50} D (140.3 mg/ kg b.wt.) in alternative days for 30 days in combination with daily prior 4 h intubation of vit. E (24 mg/ kg B.wt.) and/ or vit. C (200 mg/ kg B.wt.) and continued also after cessation of D-exposure for further 15 days as withdrawal study.

Haematological indices of D intoxicated rats indicated significant decrease in R.B.Cs. count, PCV% and Hb content in all groups. Erythocytic

Table (1): Effect of oral intubation of difenconazole (140.3 mg/kg B.wt.) on alternative days for a period of 30 days alone or with prior daily oral administration of vit. E (24 mg/kg B.wt.) and/or vit. C (200 mg/kg B.wt.) on haematological parameters in adult albino rats and after cessation of difenconazole for 15 days.

Study	Period	Parameter / Group	R.B.Cs. ($X10^6/mm^3$)	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (g/dl)	MCHC (pg)
D-administration	15 days	G1	6.88 ± 0.29^{A}	40.12 ± 1.05^{A}	11.12 ± 0.23^{A}	58.31 ± 1.89	16.16 ± 0.98	27.72 ± 1.29
		G2	5.85 ± 0.12^{aB}	34.50 ± 1.21^{aB}	9.53 ± 0.19^{a}	59.00 ± 2.14	16.30 ± 0.71	27.62 ± 1.45
		G3	5.67 ± 0.13^{ab}	33.81 ± 1.01^{ab}	9.24 ± 0.23^{a}	59.61 ± 2.14	16.41 ± 0.71	27.34 ± 1.45
		G4	5.47 ± 0.10^{ab}	33.68 ± 1.02^{ab}	9.14 ± 0.23^{a}	61.52 ± 2.14	16.70 ± 0.71	27.15 ± 1.45
		G5	5.97 ± 0.15^{a}	36.71 ± 2.10^{a}	9.97 ± 0.51^{a}	61.52 ± 3.24	16.79 ± 0.71	27.25 ± 1.45
		F-calc.	9.125#	8.657#	9.687#	2.354	1.987	2.067
	30 days	G1	7.22 ± 0.32^{A}	41.72 ± 1.12^{A}	12.12 ± 0.26^{A}	57.76 ± 1.89	16.78 ± 0.98	29.05 ± 1.29
		G2	6.10 ± 0.12^{aB}	35.10 ± 1.21^{aB}	10.22 ± 0.19^{aB}	57.50 ± 2.14	16.74 ± 0.88	29.12 ± 1.45
		G3	6.65 ± 0.14^{ab}	38.26 ± 1.08^{ab}	11.10 ± 0.26^{ab}	57.56 ± 2.19	16.71 ± 0.71	29.01 ± 1.45
		G4	7.09 ± 0.31	40.54 ± 1.09	12.00 ± 0.26	57.59 ± 3.52	16.68 ± 0.75	29.09 ± 1.45
		G5	7.28 ± 0.16	42.11 ± 2.25	12.28 ± 0.57	57.61 ± 3.24	16.63 ± 0.93	29.11 ± 1.45
		F-calc.	11.32#	12.68#	9.465#	1.257	0.687	0.987
Withdrawal study	15 days	G1	7.08 ± 0.31^{A}	40.47 ± 1.15^{A}	11.88 ± 0.27^{A}	57.17 ± 1.89	16.78 ± 0.98	29.35 ± 1.29
		G2	6.99 ± 0.15^{aB}	38.09 ± 1.16^{aB}	11.32 ± 0.28^{aB}	54.53 ± 2.14	16.20 ± 0.71	29.71 ± 1.45
		G3	6.05 ± 0.12^{ab}	33.01 ± 1.21^{ab}	9.81 ± 0.19^{ab}	54.53 ± 2.14	16.20 ± 0.71	29.77 ± 1.45
		G4	7.00 ± 0.52^{b}	38.48 ± 1.17^{b}	11.54 ± 0.31^{b}	54.97 ± 2.14	16.49 ± 0.71	30.00 ± 1.45
		G5	7.15 ± 0.28^{b}	40.97 ± 1.40^{b}	11.99 ± 0.61^{b}	57.28 ± 3.24	16.83 ± 0.71	29.38 ± 1.45
		F-calc.	10.354#	9.654#	8.975#	1.658	0.687	1.354

D = Difenoconazole
Data represented as mean ± S.E.
G1 = Control group
G2 = Group dosed D alone
G3 = Group dosed D + vit. E
G4 = Group dosed D + vit. C
G5 = Group dosed D + vit. E & C
# Significant at $P < 0.05$ using ANOVA test.
Aa, Bb significantly different between two comparison groups in the same row against capital litter at $P < 0.05$ using Least Significant Difference (LSD).

Table (2): Effect of oral intubation of difenoconazole (140.3 mg/kg B.wt.) on alternative days for a period of 30 days alone or with prior oral administration of vit. E (24 mg/kg B.wt.) and/or vit. C (200 mg/kg B.wt.) on total and differential leucocytic counts ($x10^3/mm^3$) in adult albino rats and after cessation of difenconazole for 15 days.

Study	Period	Parameter / Group	W.B.Cs.	Neutrophils	Lymphocytes	Monocytes	Eosinophils	Basophils
D-administration	15 days	G1	11.59 ± 0.297^{A}	2.646 ± 0.068^{A}	8.534 ± 0.219^{A}	0.229 ± 0.015	0.14 ± 0.007	0.041 ± 0.002
		G2	15.83 ± 0.417^{aB}	2.12 ± 0.056^{aB}	13.299 ± 0.350^{aB}	0.241 ± 0.03	0.13 ± 0.016	0.042 ± 0.005
		G3	16.1 ± 0.393^{ab}	2.09 ± 0.051^{ab}	13.604 ± 0.332^{ab}	0.239 ± 0.03	0.13 ± 0.022	0.037 ± 0.006
		G4	15.7 ± 0.393^{ab}	2.09 ± 0.052^{ab}	13.201 ± 0.330^{ab}	0.251 ± 0.042	0.12 ± 0.01	0.038 ± 0.003
		G5	16.2 ± 0.415^{a}	1.95 ± 0.050^{a}	13.841 ±0.355^{a}	0.241 ± 0.02	0.13 ± 0.01	0.038 ± 0.003
		F-calc.	11.254#	9.5421#	12.654#	2.654	1.324	0.287
	30 days	G1	11.41 ± 0.285^{A}	2.378 ± 0.059^{A}	8.639 ± 0.216^{A}	0.223 ± 0.028	0.13 ± 0.011	0.04 ±0 .003
		G2	15.41 ± 0.395^{a}	1.95 ± 0.050^{a}	13.033 ± 0.334^{a}	0.246 ± 0.041	0.14 ± 0.011	0.041 ± 0.003
		G3	15.4 ± 0.405^{a}	1.92 ± 0.051^{a}	13.074 ± 0.344^{a}	0.228 ± 0.018	0.14 ± 0.013	0.038 ± 0.003
		G4	15.3 ± 0.373^{a}	2.12 ± 0.052^{a}	12.759 ± 0.311^{a}	0.248 ± 0.023	0.14 ± 0.009	0.033 ± 0.002
		G5	15.9 ± 0.398^{a}	2.1 ± 0.053^{a}	13.387 ± 0.335^{a}	0.235 ± 0.016	0.14 ± 0.018	0.038 ± 0.005
		F-calc.	10.254#	13.254#	14.654#	1.987	1.3654	0.845
Withdrawal study	15 days	G1	11.37 ± 0.277^{A}	2.598 ± 0.063^{A}	8.418 ± 0.205^{A}	0.195 ± 0.015	0.12 ± 0.008	0.039 ± 0.003
		G2	13.25 ± 0.331^{a}	2.15 ± 0.054^{a}	10.684 ± 0.267^{a}	0.248 ± 0.017	0.13 ± 0.016	0.038 ± 0.005
		G3	14.8 ± 0.379^{a}	2.16 ± 0.055^{a}	12.25 ± 0.314^{a}	0.219 ± 0.027	0.13 ± 0.007	0.041 ± 0.002
		G4	13.1 ± 0.345^{a}	2.09 ± 0.055^{a}	10.61 ± 0.279^{a}	0.233 ± 0.012	0.13 ± 0.016	0.037 ± 0.005
		G5	12.9 ± 0.315^{a}	2.11 ± 0.051^{a}	10.396 ± 0.254^{a}	0.211 ± 0.026	0.15 ± 0.025	0.033 ± 0.006
		F-calc.	12.254#	16.542#	14.658#	0.657	0.987	1.687

D = Difenoconazole Data represented as mean ± S.E. G1 = Control group G2 = Group dosed D alone
G3 = Group dosed D + vit. E G4 = Group dosed D + vit. C G5 = Group dosed D + vit. E & C
# Significant at P < 0.05 using ANOVA test.
Aa, Bb significantly different between two comparison groups in the same row against capital litter at P < 0.05 using Least Significant Difference (LSD).

Table (3): Effect of daily oral intubation of difenoconazole on alternative days (140.3 mg/kg B.wt.) alone or with prior oral administration of vit. E (24 mg/kg B.wt.) and/or vit. C (200 mg/kg B.wt.) on some biomedical parameters of adult albino rats for 30 days and after cessation of difenoconazole for 15 days.

Study	Period	Parameter / group	T.P. (g/dl)	ALT (u/l)	AST (u/l)	Urea (mg/dl)	Creatinine (mg/dl)
D-administration	15 days	G1	5.72 ± 0.09^{A}	28.33 ± 1.12^{A}	36.67 ± 1.18^{A}	33.0 ± 1.65^{A}	0.71 ± 0.05^{A}
		G2	5.02 ± 0.28^{aB}	88.1 ± 2.65^{aB}	97.2 ± 2.12^{aB}	47.1 ±2.1^{aB}	1.9 ± 0.12^{a}
		G3	5.35 ± 0.13^{ab}	55.1 ± 1.15^{ab}	68.2 ± 1.59^{ab}	38.4 ±1.33^{ab}	1.87 ± 0.18^{a}
		G4	5.29 ± 0.19^{ab}	62.1 ± 1.09^{ab}	65.2 ± 2.1^{ab}	39.6 ± 1.65^{ab}	1.81 ± 0.09^{a}
		G5	5.31 ± 0.24^{ab}	49.1 ± 1.8^{ab}	61.9 ± 2.84^{ab}	38.7 ± 1.32^{ab}	1.79 ± 0.12^{a}
		F-calc.	9.325#	10.325#	18.9784#	11.325#	9.654#
	30 days	G1	5.79 ± 0.27^{A}	27.6 ±1.09^{A}	37.1 ± 2.01^{A}	32.8 ± 1.87^{A}	0.68 ± 0.06^{A}
		G2	4.78 ± 0.39^{a}	93.2 ± 3.12^{aB}	105.1 ± 4.18^{aB}	49.5 ± 2.9^{aB}	2.21 ± 0.15^{aB}
		G3	5.44 ± 0.21^{a}	60.2 ± 2.25^{ab}	42.1 ± 1.59ab	42.2 ± 1.65^{ab}	1.42 ± 0.17^{ab}
		G4	5.36 ± 0.20^{a}	62.9 ± 1.19^{ab}	45.9 ± 1.9^{ab}	43.8 ± 1.71^{ab}	1.39 ± 0.11^{ab}
		G5	5.66 ± 0.38	66.1 ± 1.18^{ab}	38.7 ± 1.54	33.9 ± 1.57	1.21 ± 0.19^{ab}
		F-calc.	6.254#	11.234#	8.985#	10.325#	9.685#
Withdrawal study (with vitamins)	15 days	G1	5.78 ± 0.125^{A}	28.9 ±1.23^{A}	36.14 ± 1.18^{A}	33.4 ± 1.57^{A}	0.69 ± 0.07^{A}
		G2	4.51 ± 0.23^{a}	99.6 ± 3.25^{a}	112.2 ± 4.91^{a}	53.6 ± 3.1^{aB}	2.95 ± 0.17^{aB}
		G3	5.69 ± 0.22	27.8 ± 2.1	37.6 ± 1.57	43.5 ± 1.15^{ab}	0.99 ± 0.23^{ab}
		G4	5.67 ± 0.23	29.8 ±1.15	37.9 ± 1.62	44.9 ± 1.64^{ab}	0.91 ± 0.13^{ab}
		G5	5.71 ± 0.29	30.2 ± 0.95	38.9 ± 1.65	32.8 ± 1.66	0.74 ± 0.15
		F-calc.	4.657#	5.215#	4.366#	7.654#	9.611#

D = Difenoconazole Data represented as mean ± S.E. G1 = Control group
G2 = Group dosed D alone G3 = Group dosed D + vit. E G4 = Group dosed D + vit. C
G5 = Group dosed D + vit. E & C
# Significant at P < 0.05 using ANOVA test.
Aa, Bb significantly different between two comparison groups in the same row against capital litter at P < 0.05 using Least Significant Difference (LSD).

Table (4): Effect of oral intubation of difenconazole (140.3 mg/kg B.wt.) on alternative days for a period of 30 days alone or with prior daily oral administration of vit. E (24 mg/kg B.wt.) and/or vit. C (200 mg/kg B.wt.) on serum cholesterol and triglycerides in adult albino rats and after cessation of difenoconazole for 15 days.

Study	Period	Parameter / group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
D-administration	15 days	G1	158.8 ± 4.23^{A}	115.75 ± 3.16^{A}	287.25 ± 11.14^{A}	177.12 ± 4.20
		G2	249.2 ± 8.45^{aB}	92.60 ± 5.12^{aB}	209.10 ± 8.70^{aB}	195.1 ± 5.10
		G3	191.2 ± 0.027^{ab}	104.75 ± 8.70^{b}	305.10 ± 9.14^{b}	181.2 ± 6.40
		G4	193.7 ± 0.030^{ab}	110.18 ± 8.29^{b}	301.4 ± 8.22^{b}	187.4 ± 7.10
		G5	186.8 ± 0.032^{ab}	102.14 ± 8.01^{b}	300.6 ± 7.15^{b}	175.1 ± 8.20
		F-calc.	13.254#	11.325#	10.124#	2.312
	30 days	G1	160.5 ± 4.66^{A}	120.00± 3.63^{A}	290.17 ± 18.7^{A}	150.81 ± 5.60^{A}
		G2	269.8 ± 2.99^{aB}	82.20 ± 4.89^{aB}	204.00 ± 11.6^{aB}	214.30 ± 6.10^{aB}
		G3	188.7 ± 0.032^{ab}	120.44 ± 1.98^{b}	317.31 ± 18.4^{b}	170.4 ± 3.90^{b}
		G4	181.9 ± 0.030^{ab}	111.00 ± 7.04^{b}	315.24 ± 19.6^{b}	175.3 ± 4.10^{b}
		G5	172.3 ± 0.031^{ab}	113.20 ± 3.32^{b}	305.65 ± 19.4^{b}	160.4 ± 3.80b
		F-calc.	12.542 #	11.325#	9.345#	11.342#
Withdrawal study (with vitamins)	15 days	G1	158.9 ± 4.45^{A}	108.00 ± 5.68^{A}	295.17 ± 9.14^{A}	164.11 ± 5.80^{A}
		G2	291.8 ± 13.8^{aB}	88.70 ± 4.10^{aB}	246.11 ± 12.16^{a}	191.11 ± 4.82^{aB}
		G3	165.8 ± 3.84^{b}	103.48 ± 8.07^{b}	301.14 ± 7.80	165.22 ± 4.11^{b}
		G4	171.1 ± 5.01^{b}	109.40 ± 5.95^{b}	305.18 ± 11.10	170.4 ± 3.91^{b}
		G5	173.8 ± 4.51^{b}	106.60 ± 7.58^{b}	304.06 ± 8.72	165.17 ± 3.10^{b}
		F-calc.	9.874#	21.324#	8.456#	9.786#

D = Difenoconazole Data represented as mean ± S.E.
G1 = Control group G2 = Group dosed D alone
G3 = Group dosed D + vit. E G4 = Group dosed D + vit. C
G5 = Group dosed D+ vit. E & C # Significant at P < 0.05 using ANOVA test.
Aa, Bb significantly different between two comparison groups in the same row against capital litter at P < 0.05 using Least Significant Difference (LSD).

Table (5): Effect of oral intubation of difenconazole (140.3 mg/kg B.wt.) on alternative days for a period of 30 days alone or with prior oral administration of vit. E (24 mg/kg B.wt.) and/or vit. C (200 mg/kg B.wt.) on total triiodothyronin (T3) and total thyroxin (T4) in adult albino rats and after cessation of difenoconazole for 15 days.

Study	Period	Parameter / Group	T3 (ng/ml)	T4 (ng/ml)
D-administration	15 days	G1	1.814 ± 0.047^{A}	40.721 ± 1.044^{A}
		G2	1.049 ± 0.028^{a}	35.214 ± 0.927^{a}
		G3	1.112 ± 0.027^{a}	32.541 ± 0.794^{a}
		G4	1.214 ± 0.030^{a}	31.254 ± 0.781^{a}
		G5	1.265 ± 0.032^{a}	32.145 ± 0.824^{a}
		F-calc.	9.215#	12.325#
	30 days	G1	1.821 ± 0.046^{A}	39.214 ± 0.98^{A}
		G2	1.121 ± 0.029^{a}	33.214 ± 0.852^{a}
		G3	1.215 ± 0.032^{a}	30.254 ± 0.796^{a}
		G4	1.229 ± 0.030^{a}	32.546 ± 0.794^{a}
		G5	1.235 ± 0.031^{a}	33.298 ± 0.832^{a}
		F-calc.	10.324#	8.654#
Withdrawal study (with vitamins)	15 days	G1	1.809 ± 0.044^{A}	39.687 ± 0.968^{A}
		G2	1.321 ± 0.033^{aB}	32.654 ± 0.816^{aB}
		G3	1.308 ± 0.034^{aC}	34.214 ± 0.877^{aC}
		G4	1.811 ± 0.035^{bc}	39.254 ± 0.901^{bc}
		G5	1.823 ± 0.032^{bc}	38.656 ± 0.845^{bc}
		F-calc.	6.987#	15.254#

D = Difenoconazole Data represented as mean ± S.E.
G1 = Control group G2 = Group dosed D alone
G3 = Group dosed D + vit. E G4 = Group dosed D + vit. C
G5 = Group dosed D + vit. E & C
# Significant at $P < 0.05$ using ANOVA test.
Aa, Bb significantly different between two comparison groups in the same row against capital litter at $P < 0.05$ using Least Significant Difference (LSD).

indices did not differ significantly from controls. However, a marked elevation in W.B.Cs. and lymphocytes counts were recorded. On the other hand, neutrophils count decreased in comparison to negative controls.

Comprehensive data assessing the biological effects of D are very limited. The present results were in agreement with the effects of other triazoles compounds as reported by Lessel (1974) in rats dosed 200 mg/ kg 4- nitroaniline for 30 days and with Kinsey *et al.* (1984) in rats fed diet containing 0, 50, 500 or 5000 ppm hexaconzole for 90 days. The decline in R.B.Cs. count could be described either to destruction of cells or their enhanced removal from circulation (Nemi, 1993).

Leucocytosis was appeared as a defense mechanism against toxic substances (Marx, 1996), while neutropenia may result due to cell migration and sequestration in the microvasculature, especially in the lung. Neutroponia may also be due to endotoxine administration, which induced toxic changes as vacullation and cytoplasmic foaminess of neutrophils (Jain, 1993).

The increased lymphocytes counts could be ascribed as an immune response to antigenic stimulation (Jain, 1993).

Group administered vit. C only or in combination with vit. E showed better improvement in R.B.Cs., PCV% and Hb content after 30 days of exposure and at withdrawal period, meanwhile group given vit. E alone showed no improve-

ment in haematological parameters. These results were in accordance with (Mahmoud, 2004) in small and big fishes. These findings may be due to antagonistic effect of vit. C against poisoning, vit. C also protects red cells from being destroyed by toxic substances (Wu *et al.*, 2002).

Difenoconazole decreased serum total protein in all groups allover the experimental period except group 5 that received vit. E and vit. C. These results agreed with that reported by Kinsey *et al.* (1984) with hexaconazole in male rats.

The reduction in total protein level may be due to hepatotoxic effects of fungicides with subsequent impaired synthesis of albumin and consequently total protein by the damaged liver cells (Deoras *et al.*, 1997).

Protein levels in rats given vit. E and/or vit. C showed normal values. These results suggested that vit. E and vit. C improve the synthesis of specific protein and enzymes required in differentiation or adaptation of given tissues (Diehl and Delincee, 1986).

AST and ALT levels recorded significant rise in all groups at 15 days of D exposure. Similar results were noticed by Kinsey *et al.* (1984) and Hext (1988) in rats by hexaconazole and Stonard (1989) in dogs administered different doses of hexaconazole. The hyperactivity of AST and ALT could be attributed to drastic effects caused by fungicides administration and may provoke hepatic cells as liver is the target organ of detoxicosis (Linne and Ringsrude, 1993). Activity of AST returned to normal values in groups given D with vit E and vit C after 30 days of experiment, meanwhile groups received vit. E and/ or vit. C with D showed gradual integrity in their levels at withdrawal period. The protective effects of vit. E and/ or vit. C on AST and ALT values may be related to that vit. E induced regeneration of hepatocytes with subsequent increase in the catabolism of enzymes in blood plasma (Zilva *et al.*, 1988).

Vit. C also stimulates hepatic microsomal enzyme activity, and controls the oxide-reduction activities in hepatic cells (MacCay and King, 1980).

Kidney function tests revealed an increase in serum urea and creatinine levels in D-administered rats, whereas urea recorded normal value in groups given vit. E combined with vit. C at day 30 of experiment and also at the withdrawal period, however creatinine level showed normal level in group taken vit. E and vit. C after stopping D administration.

Little studies traced the effect of D on kidney function, but the obtained results were in agreement with that of other fungicides as reported by Rosner *et al.* (1996) who used chlotholonil in rats and Kellner *et al.* (1997) in fisher rats by 3, 5 dichlorphenon. Such renal dysfunctions may be attributed to corrupted renal glomerular filtration rate (Lees *et al.*, 1994 and Kaneko *et al.*, 1997).

Recovey effects of vit. E and vit. C may be attributed to their enhanced effects of antioxidants on tissues. They also improved circulation and counteract nephrotoxicity (Basu and Schoroch, 1982 and Traber and Packer, 1995).

Regarding cholesterol level the present investigation showed pronounced rise in its level in all groups allover the experimental period while its level regressed to become normally in groups given vit. E and/ or vit. C at withdrawal period.

LDL levels were elevated in group exposed to D only from the beginning till the end of experiment and also after cessation of D administration. Meanwhile other groups that received D as well as antioxidant drugs recorded rapid response from day 15 till day 30 of experiment and at withdrawal study. However triglyceride (TG) and HDL recorded marked reduction in their levels in the group intubated D only, they returned to normal values in other groups that given antioxidant drugs from the beginning till the end of experiment and also at the withdrawal period. Results cholesterol and T.G. in the present results agree with the finding of Hext, (1988) and Stonard, (1989) who studied the effect of hexaconazole in rats and dogs respectively. An increase in circulating cholesterol and LDL is almost due to lack of LDL uptake via receptor dependent pathway and/or hypothyrodiam that caused an increase in the level of LDL in blood (Zilva *et al.*, 1988), whereas the marked reduction in the level of TG may be due to either decrease of the level of very LDL, chylomicrones or both, as a source of T.Gs. which could be attributed to the activation of triglyceride lipase in treated rats (Stein, 1996). Decreased HDL levels in D treated group may be due to impairment in the biosynthesis of HDL in hepatic and intestinal cells. Moreover HDL is important in the removal of cholesterol from the cells (Zilva *et al.*, 1988).

A significant reduction in levels of cholesterol and LDL nearly to normal in groups administered antioxidant drugs is in agreement with (Hokkaido, 1976). These results may be attributed to the effect of vit. E that appeared to depend upon the suppression of cholesterol biosynthesis and increase of cholesterol catabolism as well as the excretion of bile acid (Sun *et al.*, 1997). Vit. C also stimulates cholesterol-7-alpha hydroxylase enzyme, which regulates the conversion of cholesterol to bile acid; a vehicle for cholesterol excretions (Halaway and Rivers, 1981).

The present study showed a significant decease in (T_3) and (T_4) levels in D treated groups all over the experiment and at withdrawal period. The present results were in agreement with those of (Nebbia *et al.* (1991); WHO, (1994); Eman (2004) and Mahjoŭbi *et al.* (2005) on calves administered zinc ethylene- bio-dithiocarbamate; in rats exposed to different doses of amitrole; by using diviconazole in rats in female rats injected dimethoate. The reduction in (T_3) and (T_4) levels may be due to impairment in the biosynthethesis of thyroid hormones in cells of thyroid gland due to direct action of fungicide (Van-Lee Ween, 1996). Meanwhile, levels of these hormones became within normal levels in groups administered vit. C alone or with vit. E these results could be attributed to that vit. C helps in biosynthesis of thyroid hormones by restoring the epithelial follicular cells of thyroid gland (Williams, 1993).

The results of the present study indicate that vit. C alone induced an early response than vit. E in R.B.C's., PCV% and Hb content after 30 days of D exposure while in (T_3) and (T_4) after stopping D administration at withdrawal time. On the other hand, vit. E and vit. C have the same positive effect on other biochemical parameters at withdrawal period, but combination of these vitamins with each other produce wider protective activity in all tested parameters especially after 30 days of D exposure.

In conclusion, the current study indicated that difenoconazole administration provokes direct and rapid toxic effects on blood picture, liver and kidney activities as well as thyroid function, which could be suppressed or protected by using vit. E and/or vit. C as antioxidant drugs.

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