

Potential protective effects of vitamin E on diazinon-induced DNA damage and some haematological and biochemical alterations in rats

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Abstract

Diazinon (DZN), is a commonly used organophosphorous (OP) pesticide to control a variety of insects in agriculture and in the environment. Vitamin E is a primary antioxidant that plays an important role in protecting cells against toxicity by inactivating free radicals generated following pesticides exposure. Therefore, the present study was undertaken to investigate the possible protective effect of vitamin E against DZN- induced adverse effects on haematological and biochemical indices and on genotoxicity using comet assay and micronucleus test for measuring the DNA damage. The tissue DZN residues in liver, kidney and muscle of the rats were determined. Vitamin E (200 mg/kg, twice a week), diazinon (10mg/kg/day, once a day), and vitamin E (200mg/kg, twice a week + diazinon (10mg/kg/day, once a day) combination were given to rats (n= 10) orally via gavage for 4 weeks. The results revealed that DZN administration significantly decreased Hb concentration, RBCs count and PCV values. Meanwhile, a significant increased in WBCs, ALT, AST and total cholesterol was detected. However, vitamin E supplementation together with DZN improves these alterations. DZN residues level is highest in the kidney than that in liver and muscle tissues. Administration of vitamin E together with DZN reduces the residual values in the examined tissues. A significant increase in tail length of comets from blood cells as well in the frequency of micronucleated cells (MNCs) following DZN administration was achieved. Co-administrated vitamin E along with DZN resulted in decrease in tail length of comets and the percentage of MNCs compared to DZN alone treated rats. The increase in frequency of MNCs and tail length of comets confirm the genotoxicity of DZN. Vitamin E, on the other hand, was observed to repair the genotoxicity and improves the haematological and biochemical changes induced by DZN. It can be concluded that vitamin E has protective effect against DZN adverse effects and supplementation of vitamin E might be beneficial to DZN exposed populations.

Introduction

Diazinon (DZN) is a commonly used organophosphorous (OP) pesticide (diethoxy- [(2-isopropyl-6-methyl-4-pyrimidinyl) oxy]-thioxophosphorane). It is a synthetic chemical substance with broad

spectrum insecticide activity [1]. Toxic effects of diazinon are due to the inhibition of acetylcholinesterase activity, an enzyme needed for proper nervous system function. It has been widely used throughout the world with applications in agriculture and horticulture for controlling insects in crops,

ornamentals, lawns, fruit, vegetables and other food products [2,3]. Some reports have been published with respect to DZN and its effects on haematological and biochemical parameters of rat, rabbits and mice [4-8]. Toxicities of OP insecticide DZN cause adverse effects on many organs [5]. Other systems that could be affected are immune system [9], urinary system [10], reproductive system [11], pancreas [12] and liver [5]. The mild structural and functional changes in the liver as well as in the kidney were observed in mice after 14 days of intraperitoneal (i. p) injection of 1/4 and 1/2 LD50 of DZN [8]. DZN may interfere with lipid metabolism in mammalian animals that included the levels of total cholesterol, high –density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and phospholipids, and its effect was dose-dependent [9].

Diazinon's mutagenicity studies, its ability to cause genetic damage, showed that DZN in fact can damage DNA in human blood cells, in cells from laboratory animals, and in bacteria [13]. DZN exposure was found to increase the occurrence of a type of genetic damage called micronuclei (MN). Micronuclei may be induced by strand breaks in DNA due to oxidative stress [14]. The MN test using peripheral blood cells is used to detect chromosome breaks that derive from DNA damage [15].

Many insecticides such as DZN are hydrophobic molecules, which bind extensively to biological membranes, especially to the phospholipids bilayers. Vitamin E (α -tocopherol) is a natural component of the membrane lipid bilayer. Studies carried out with antioxidants such as vitamin E, have shown that they scavenged and inhibit free radicals formation [11], and may effectively minimize lipid peroxidation in biological systems. It was reported that vitamin E decreased DZN genotoxicity and protected some of the biochemical indices [6].

Therefore, the present study was undertaken to evaluate the possible beneficial effect of vitamin E against DZN-induced genotoxicity using comet assay, measuring the DNA damage, and micronucleus test as a good indicator for strand breaks in DNA and the induction of chromosomal aberrations [12]. As well, some haematological indices, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine and cholesterol levels and the tissue residual in various body organs (liver, kidney and muscle tissues) of rats were evaluated.

Materials and Methods

Chemicals

Diazinon, was obtained from ADWIA 60% EC (Emulsifiable concentrate), Cairo, Egypt. Vitamin E (DL- α -tocopherol acetate) was supplied by Merck Ltd., SRL Pvt. , Ltd. , Mumbai, India.

Animals and treatment

Male Wistar rats, weighting about 230-250gm, obtained from the laboratory animal house of the National Research Center, Dokki, Cairo, Egypt were used in the study, as males have been shown to be more susceptible than females to genotoxic effects of various chemical [16]. The animals were housed in polypropylene cages, given water *ad libitum* and fed standard pellet diet for 2 weeks for adaptation. Rats were exposed to a 12h light: 12h dark cycle, at a room temperature of 18-22°C, and segregated into four groups, each group having 10 animals. Animals were administered orally DZN and/or vit. E orally for a period of 4 weeks as follow: 1- Animals in the control group received 1 ml of water daily, orally. 2- Vitamin E treated animals were administered vitamin E (200mg/kg bw), twice a week. 3- Diazinon treated group was given DZN (10 mg/kg bw/day) and 4- Animals in the DZN + vit. E treated group received of DZN (10mg/kg bw/day) and vit. E (200mg/kg bw) twice a week, orally [6].

Collection of blood samples

Rats were anaesthetized by diethyl ether, blood samples were taken from retro orbital venous plexus using glass capillaries. Blood samples were collected weekly for haematological and biochemical examination. For comet assay and micronucleus test, blood samples were collected at the end of the 2nd and the 4th week of the experiment.

Haematological examination

Blood samples with anti-coagulant EDTA were analyzed for haematological parameters [red blood cells (RBCs) count, hemoglobin (Hb), packed cell volume (PCV) and white blood cells (WBCs) count] [17].

Biochemical evaluation

Serum samples were obtained for spectrophotometric determination of total protein, albumin, ALT, AST, urea, creatinine and total cholesterol using kits purchased from bio Merieux Sa-69280 Marcy L'Etoile, France.

Genetic analysis

Comet assay

Comet assay was performed under alkaline conditions [12] with slight modification. Slides were scored using Comet Score Software (Tritek Corp. , USA) and 50 cells were analyzed per sample. The parameters used to assess DNA damage were tail length (migration of DNA from nucleus) and tail moment which was automatically generated by comet score software. The control and treated slides were randomized and were not run separately or at different times to avoid variability.

Micronucleus test

Micronucleus test was performed [15], with slight modification. A small drop of blood was placed at one end of clean, grease free microscopic slide. The drop was carefully spread into a single cell layered film without damaging the cell morphology using a polished cover glass held at an angle of 45°. The slides were air dried for 12 h and subsequently stained for 1-2 min in concentrated May- Grunwald stain (0. 25% in methanol) followed by 10% Giemsa stain solution for 10min [18]. The slides were then rinsed twice with distilled water, dried and rinsed with methanol. The slides were placed in xylene for clearing, mounted in DPX and analyzed for the

presence of micronuclei (a total of 1000 cells were scored for each animal).

Residue in tissues

Samples from liver, kidney and muscles were collected at the end of the experiment to determine DZN residue according to [19].

Statistical analysis

The obtained data were subjected to one –way analysis of variance (ANOVA) using SAS program [20], followed by U-test [21] for multiple range comparison between groups. Values with $P \leq 0. 05$ were considered as significant.

Results and Discussion

The results of the present study revealed a significant decrease in Hb concentration, RBCs count and PCV values in DZN administered group from the 3rd week of experiment compared to control and vit. E treated groups. Earlier decrease in Hb was observed from the 1st week followed by a decrease in both Hb and RBCs values in the 2nd week [6,7]. Administration of vit. E/diazinon improves Hb concentration, RBCs count and PCV values (Table 1).

Table 1: Haematological parameters of control, vitamin E and/or diazinon exposed rats.

Parameters	Control	Vitamin E	Diazinon	Vitamin E + Diazinon
1st week				
RBCs x10 ⁶	7.27±0.26	7.21±0.37	7.37±0.18	7.11±0.23
Hg g/dl	13.46 ^a ±0.08	12.27 ^{ab} ±0.26	11.65 ^b ±0.57	12.51 ^{ab} ±0.29
PCV %	41.00±0.45	41.20±0.58	40.80±0.37	40.8±0.73
WBCs x10 ³	10.35±0.72	10.96±0.47	10.15±0.42	8.99±0.40
2nd week				
RBCs x10 ⁶	7.55 ^a ±0.25	7.85 ^a ±0.05	6.12 ^b ±0.39	7.93 ^a ±0.16
Hg g/dl	14.29 ^a ±0.56	13.04 ^{ab} ±0.46	12.20 ^b ±0.38	13.91 ^{ab} ±0.59
PCV %	42.80±1.18	40.00±0.32	43.60±1.33	41.00±1.43
WBCs x10 ³	8.26 ^b ±0.97	10.42 ^b ±0.29	12.64 ^a ±0.86	9.03 ^b ±0.12
3rd week				
RBCs x10 ⁶	6.99 ^a ±0.24	7.32 ^a ±0.10	5.66 ^b ±0.32	6.72 ^a ±0.30
Hg g/dl	14.43 ^a ±0.16	14.64 ^a ±0.17	12.98 ^b ±0.39	14.01 ^a ±0.17
PCV %	43.20 ^a ±0.58	42.20 ^{ab} ±0.37	39.88 ^c ±0.64	40.60 ^{bc} ±0.66
WBCs x10 ³	10.20 ^b ±0.44	11.81 ^b ±0.62	14.57 ^a ±0.64	11.68 ^b ±1.12
4th week				
RBCs x10 ⁶	6.97 ^a ±0.25	6.30 ^{ab} ±0.10	5.16 ^c ±0.15	6.12 ^b ±0.14
Hg g/dl	14.31 ^a ±0.19	14.01 ^a ±0.13	12.50 ^b ±0.55	13.98 ^a ±0.24
PCV %	45.20 ^a ±0.66	44.60 ^a ±0.51	39.60 ^c ±0.68	42.00 ^b ±0.71
WBCs x10 ³	9.82 ^b ±0.21	8.18 ^c ±0.24	13.03 ^a ±0.51	8.91 ^{bc} ±0.23

Values represents means ± standard errors (SE). Number of animals/group = 5

Values in the same raw with different superscript letters are differing significantly ($p < 0. 05$).

Slight improve in PCV values that did not reach the PCV values of control group were observed from 3rd and 4th week of experiment. The decrease in Hb concentration along with the decrease in RBCs count might be due to the effect of pesticide on erythropoietic tissue [6]. Pesticide residues play a role in the development of anemia due to interference of Hb biosynthesis and shorting of the life span of the circulating erythrocyte [22].

Significant increase in total leucocytic count was observed from the 2nd week till the end of experiment in the DZN administered group compared to control and vit. E treated groups. Vit. E/DZN administration significantly decreased the elevating WBCs count till

the end of experiment (Table 1), where the WBCs count nearly coincide with that of the control group and the group given vit. E only. This result agreed with [23], who found significant increase in total leucocytic count in female mice. This increase in leucocytic count may indicate an activation of animal defense mechanism and immune system [6].

The improved change in haematological parameters in the group given vit. E as a protective could be due to the fact that vit. E neutralizes lipid peroxidation and unsaturated membrane lipids because of its oxygen scavenging effect [6,11,24,25] thus preventing the possible tissue damage caused by the pesticide.

Table 2: Some biochemical parameters of control, vitamin E and/or DZN exposed rats.

Parameters	Control	Vitamin E	Diazinon	Vitamin E + Diazinon
1st week				
ALT (u/l)	43.82 ± 0.81	43.16 ± 0.52	45.73 ± 0.59	42.6 ± 1.00
AST (u/l)	67.48 ± 0.25	67.44 ± 1.38	68.62 ± 1.52	67.42 ± 0.66
Cholesterol (mg/dl)	40.68 ^b ± 1.43	39.00 ^b ± 0.91	51.48 ^a ± 2.65	42.33 ^b ± 0.76
Urea (mg/dl)	16.66 ± 0.74	17.16 ± 1.02	14.56 ± 0.75	14.74 ± 0.17
Total protein (g/dl)	6.64 ± 0.23	7.18 ± 0.30	7.00 ± 0.32	7.60 ± 0.34
Albumin (g/dl)	3.48 ± 0.13	3.12 ± 0.26	3.24 ± 0.46	3.56 ± 0.04
Creatinine (mg/dl)	1.12 ± 0.02	1.10 ± 0.02	1.20 ± 0.05	1.10 ± 0.02
2nd week				
ALT (u/l)	41.48 ^b ± 0.49	41.08 ^b ± 0.43	44.04 ^a ± 0.45	41.49 ^b ± 0.43
AST (u/l)	60.52 ^b ± 0.68	57.80 ^b ± 0.61	66.34 ^a ± 2.49	60.02 ^b ± 1.32
Cholesterol (mg/dl)	52.53 ^b ± 2.75	50.07 ^b ± 1.27	74.62 ^a ± 2.32	44.90 ^b ± 4.26
Urea (mg/dl)	22.12 ± 1.98	21.64 ± 2.28	28.58 ± 2.30	18.92 ± 2.59
Total protein (g/dl)	6.68 ± 0.28	6.70 ± 0.21	7.10 ± 0.36	6.44 ± 0.33
Albumin (g/dl)	3.52 ± 0.38	3.26 ± 0.11	3.14 ± 0.45	3.28 ± 0.26
Creatinine (mg/dl)	1.11 ± 0.02	1.10 ± 0.01	1.09 ± 0.01	1.05 ± 0.01
3rd week				
ALT (u/l)	41.00 ^b ± 0.41	41.20 ^b ± 0.61	43.76 ^a ± 0.45	42.10 ^b ± 0.40
AST (u/l)	60.32 ^b ± 0.62	61.50 ^b ± 0.54	64.74 ^a ± 0.84	59.86 ^b ± 1.09
Cholesterol (mg/dl)	43.50 ^b ± 1.25	37.20 ^b ± 0.44	61.22 ^a ± 1.59	38.30 ^b ± 2.95
Urea (mg/dl)	15.68 ± 0.65	15.60 ± 0.41	14.08 ± 0.67	16.00 ± 0.61
Total protein (g/dl)	6.93 ± 0.09	6.30 ± 0.29	6.04 ± 0.25	6.25 ± 0.25
Albumin (g/dl)	3.60 ± 0.04	3.50 ± 0.15	3.08 ± 0.34	3.20 ± 0.48
Creatinine (mg/dl)	1.10 ± 0.03	1.09 ± 0.02	1.17 ± 0.02	1.15 ± 0.02
4th week				
ALT (u/l)	41.86 ^b ± 0.49	42.46 ^b ± 0.50	44.38 ^a ± 0.77	40.90 ^b ± 0.52
AST (u/l)	59.60 ^b ± 0.79	61.63 ^b ± 0.73	64.12 ^a ± 0.99	61.06 ^b ± 0.34
Cholesterol (mg/dl)	46.95 ^b ± 0.94	46.82 ^b ± 1.07	56.22 ^a ± 2.17	47.96 ^b ± 0.52
Urea (mg/dl)	16.78 ± 0.39	21.36 ± 1.32	16.88 ± 2.26	20.94 ± 1.89
Total protein (g/dl)	5.78 ± 0.30	5.93 ± 0.22	5.68 ± 0.36	5.34 ± 0.24
Albumin (g/dl)	3.48 ± 0.41	3.53 ± 0.42	2.65 ± 0.37	3.58 ± 0.29
Creatinine (mg/dl)	1.13 ± 0.04	1.12 ± 0.01	1.12 ± 0.02	1.16 ± 0.03

Values represents means ± standard errors. Number of animals/group = 5

Values in the same raw with different superscript letters are differing significantly (p<0. 05).

Significant increase in ALT and Ast were detected from the 2nd till 4th week in the diazinon exposed rats (Table 2). The ALT and AST activities of vit.E/ DZN group were coincide with those of the control and vit. E treated groups. The increased ALT and AST values agreed with the results obtained [5] who stated that changes in these enzymes level might differ depending on exposure time and dose, as well as to [23] results who found that administration of vit. E/DZN improves the activity of ALT and AST in mice.

ALT and AST are important indicators of liver damage in clinic finding. These enzymes were secreted to blood in hepatocellular injury and their levels increased [5]. The liver cells are the sites of toxic action of DZN [26], which affects mitochondrial membrane transportation in liver [27-29] and caused swelling of mitochondria in hepatocytes [5], resulting in increased of biochemical indices and liver enzymes.

Significant increase in serum cholesterol in the DZN administered group started from the first week till the end of experiment compared to control and vit. E treated groups (Table 2)[5,7]. Vit. E administration together with DZN lowers significantly the cholesterol level approximately to that of control and the vitamin E only treated groups (Table 2)[5]. The significant increased in serum cholesterol levels can be attributed to the effect of pesticide on the permeability of liver cell membrane [5] and may be also attributed to the partial blockage of liver bile ducts causing cessation of its excretion to the duodenum [30].

In general, pesticide intoxication produces oxidative stress by the generation of free radicals and induced tissue lipid peroxidation in mammals and other organisms [31,32]. Thus the use of vitamin E as a protective antioxidant during DZN administration helps to maintain membrane stability [33]. Vitamin E allows free radicals to reduce a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids thus breaking the chain of free radical reactions [34].

Diazinon residues level is highest in the kidney when comparing to DZN concentration among liver, kidney and muscle tissues (Table 3). Administration of vitamin E with DZN insignificantly reduces the residue values in the examined tissues (Table 3). The increased residual levels in the kidney than in the liver confirm that the DZN residue was much greater in kidney than that in other organs [35,36]. The relative high concentration of DZN residue in the kidney indicates that the kidney plays an essential role in the excretion of DZN. FAO [37] reported that elimination of DZN

Table 3: Diazinon residue (mg/kg) in rat kidney, muscle and liver tissues.

Groups	Kidney	Muscle	Liver
Control	N D	N D	N D
Vitamin E	N D	N D	N D
Diazinon	8.96±0.98	1.98±0.19	1.47±0.19
VitaminE + Diazinon	7.31±0.25	2.02±0.22	1.24±0.11

Values represents means ± standard errors.

N D, non-detectable.

was mainly in urine. Similar results were recorded in different animal species, where the DZN residue in sheep kidney was about 2 fold higher than that in liver after dermal treatment for 3 days with 40mg/kg bw of DZN. These results were achieved in poultry tissues as well [37].

DNA is a target for mutagens and carcinogens, which induce changes in DNA structure giving rise to mutations and/ or cell death [38]. Free radical generated following pesticide exposure may lead to extensive DNA damage [39]. DZN is capable of inducing chromosomal aberrations such as sister chromatid exchanges. In the present study DNA assay damage was evaluated by comet and micronucleus test. Administration of DZN resulted in DNA damage as is evident from Fig. 1 (C, D) correspond to DNA from animals exposed to DZN for 14 and 30 days, respectively. It is evident that exposure to DZN resulted in DNA damage as compared to control (plate A) and vitamin E treated rats (plate B). DNA damage was more pronounced after 30 days of DZN treatment. It is clear that extent of DNA damage is time dependent. Vitamin E in combination with DZN treatment reduced DZN- induced DNA damage (plates E and F). These results suggest that vitamin E has a protective effect on DZN- induced DNA [40] and showing that vitamin E prevent genotoxicity induced by DZN.

The present results (Tables 4, 5 and Fig. 1) showing the values of tail length and moment obtained

Table 4: Mean comet tail length (µm) of rat leucocytes exposed to Vit. E and/or diazinon.

Treatment	Mean comet tail length (Mean ± S. E)	
	14 Days	30 Days
Control	1.03 ^b ± 0.16	1.32 ^b ± 0.23
Vitamin E	1.43 ^b ± 0.41	1.62 ^b ± 1.15
Diazinon	2.45 ^a ± 0.31	6.75 ^a ± 0.17
Diazinon+ vitamin E	1.31 ^b ± 0.41	4.43 ^a ± 0.61

Values with different superscript letters are differing significantly. Values are expressed as mean ± S. E.

Table 5: DNA damage frequency in rat leucocytes exposed to Vitamin E and/or diazinon.

Treatments	Comet analysis	Exposure times	
		14 Days	30 Days
Control	Damage frequency (%)	1.3 ^b ± 0.9	2.8 ^b ± 5.4
Vitamin E	Damage frequency (%)	2.7 ^b ± 5.9	2.5 ^b ± 1.5
Diazinon	Damage frequency (%)	8.4 ^a ± 2.3	17.0 ^a ± 4.3
Diazinon+ vitamin E	Damage frequency (%)	6.2 ^a ± 3.5	4.4 ^b ± 3.1

Number of investigated cells = 50. Values are expressed as mean ± S. E.
Values with different superscript letters are differing significantly.

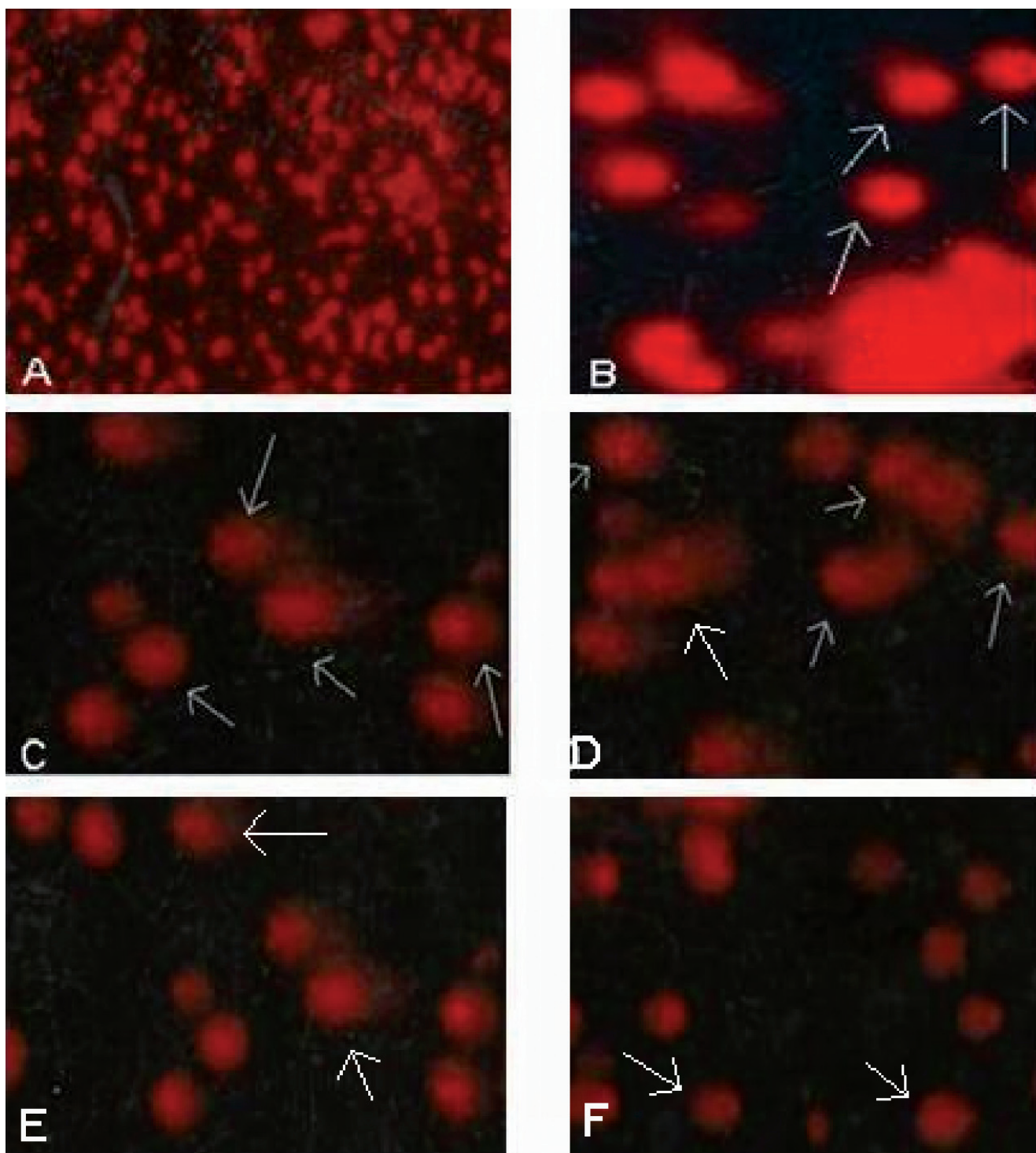


Fig. 1. Fluorescence microscopy image of leucocyte cellcomets; (A) Control untreated cells. (B) Vitamin E treated cells. (C&D) DZN only exposed for 14 and 30 days. E&F Vit.E/DZN exposed for 14 and 30 days (arrows indicate comets).

from comet assay after treatment with DZN and/or vitamin E. It is clear that there was a significant ($P < 0.05$) increase in tail length in blood cells of rats treated with DZN as compared to the control rats specially for the long period treatment (30 days), which was also illustrated by the representative comets (Fig. 1) and to our knowledge this is the first report showing the genotoxic effect of DZN using comet assay.

Data in Tables 4 and 5 showing that vitamin E had a protective effects against DZN-induced DNA damage specially in short period treatment for tail length and in long period treatment for tail moment. The protective effect of vitamin E against genotoxicity of various chemicals has been reported [40,41]. Based on the results it is clear that DZN might be inducing DNA damage by increased generation of reactive free radicals which are scavenged by vitamin E. The protective effect of vitamin E may attributed to its antioxidant action but not to its interaction with DZN.

The results of genotoxicity of DZN assessed by scoring MN (Table 6), corroborate with the findings of comet assay. A significant ($P < 0.05$) increase in the frequency of micronuclei was observed after treatment with DZN as compared to that in control. This is clear in both 2 periods (14 and 30 days) of treated groups.

The results further confirm that DZN exposure induced DNA damage. Using MN assay, DZN treatment to human blood cells (lymphocytes and erythrocytes) and skin fibroblasts has been shown to results in a significant increase in the number of MN via clastogenic mode of action, inducing single and double strand breaks on DNA molecule [42]. The clastogenic mechanism of action of organophosphate pesticides was also observed in vivo studies in mice [43]. These findings are consistent with a number of reports in which pesticide exposure has been associated with increase in MN incidence in cultured lymphocytes isolated from peripheral blood taken from exposed individuals [44,45]. However, the exposure to atrazine (OP pesticide) was ineffective in inducing clastogenic and aneugenic damage in cultured human lymphocytes,

Table 6: Micronuclei frequency (MN/1000 erythrocytes) in short (14 days) and long term (30 days) of control, vitamin E and /or diazinon exposed rats.

Treatments	Mean + S.E	
	14 Days	30 Days
Control	0.43 ^b ± 0.2	0.5 ^b ± 0.3
Vitamin E	0.53 ^b ± 0.46	0.77 ^b ± 0.3
Diazinon	5.63 ^a ± 2.1	9.2 ^a ± 4.2
Diazinon+ vitamin E	3.24 ^a ± 1.3	6.5 ^a ± 1.2

Small different superscript letters are differing significantly.

whereas, a significant increase in the frequency of chromosome aberrations in mouse bone marrow cells was observed [46].

Administration of DZN in combination with vitamin E showed decrease in the percentage of MN as compared to DZN treated rats (about 60% less than DZN exposed rats on 14 and 30 days of treatment). It is evident that vitamin E might be reducing the MN formation by scavenging the DNA damaging free radicals generated following DZN exposure. Another possible mechanism of vitamin E might involve selective removal of cells with DNA damage by apoptosis [12]. The suppressive effect of vitamin E on growth of tumor cells is due to the ability of vitamin E to induce cell cycle arrest and/ or apoptosis of transformed cells [47]. These results demonstrate that administration of vitamin E along with DZN decreased the DNA damage and thus protected the cells against genotoxic effect of DZN.

In summary our findings demonstrate that DZN is genotoxic as assessed by comet and micronucleus assays and had adverse effects on some haematological and biochemical parameters. Vitamin E, on the other hand, was observed to repair the genotoxicity and improves the haematological and biochemical changes induced by DZN. It can be concluded that vitamin E, as an antioxidant, has protective effect against DZN adverse effects by inactivating (scavenging) free radicals generated following pesticides exposure and supplementation of vitamin E might be beneficial to DZN exposed populations.

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