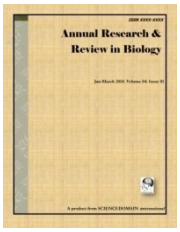

Annual Research & Review in Biology
4(24): 4023-4033, 2014

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Protective Effects of Vitamins C and E on Dimethoate- Induced Nephrotoxicity in Male Guinea Pigs

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Authors' contributions

This work was carried out in collaboration between all authors. Author YSAA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors MAAD and EMA managed the analyses of the study. Author GHES managed the literature review and refines the final draft. All authors read and approved the final manuscript.

Original Research Article

Received 31st March 2014
Accepted 8th May 2014
Published 24th July 2014

ABSTRACT

Aims: To examine the protective properties of vitamin C (vitC) and E (vitE) on DM induced nephrotoxicity in male guinea pigs.
Study Design: Twenty male rats were randomly grouped into four. "1" = Control, "2" = Vitamins (C & E) treated, "3" = Dimethoate (DM) treated and "4" = Vitamins treated plus DM treated.
Place and Duration of Study: Department of Biology, Ibb University, Ibb, Yemen between February 2011 and May 2011.
Methodology: Four animal groups were used, G1: received 1.0ml of distilled water and 1.0ml of olive oil, G2: received 200mg/kg b.w/day of vitamins (C & E), G3: was treated with 7mg/kg b.w/day DM and G4: was treated with 200mg/kg b.w/day of vitamins (C & E) plus 7mg/kg b.w/day DM. All the previous administrations were repeated daily for 28 days. At the end of the experiment, the animals were sacrificed and dissected. Blood and tissue

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samples were taken for biochemical, and light microscope investigations.
Results: DM administration resulted in a significant increase in the serum urea and creatinine levels and lipid peroxidation (LPO) level, while it caused significant decreases in the activities of catalase (CAT) and glutathione-S-transferase (GST). In contrast, co-administration of vitC and vitE to DM-treated animals restored most of these biochemical parameters to nearly normal levels. Also, DM induced histopathological changes in the kidneys of all treatment groups but these alterations were predominant in DM-treated group.
Conclusion: The results showed that co-treatment of vitE and vitC may protect the guinea pigs from DM-induced nephrotoxicity.

Keywords: Dimethoate; nephrotoxicity; vitamins; guinea pigs.

1. INTRODUCTION

A pesticide is defined as any substance intended for preventing, destroying, or controlling any pest – including vectors of human or animal disease [1]. A wide range of different pesticides are commonly used in agriculture to enhance food production, especially in the developing countries [2]. The extensive use of pesticides leads to severe environmental and health hazards to many organisms [3,4]. Organophosphorus (OP) compounds are by far the most important class of pesticides, both in terms of worldwide usage and their toxicity to humans and animals [5,6]. Recently, more than 100 different OP compounds have been synthesized and are extensively used worldwide [7]. These pesticides may reach the marine environment through rivers and the atmosphere [8]. Toxicity of OP pesticides results in toxic effects on many organs and systems such as the liver, kidney, nervous system, immune system and reproductive system [9]. Dimethoate (DM), which is considered to be one of the most important OP pesticides, is commonly used in agriculture against a wide range of pests especially in Yemeni Qat farms. The residue of DM and its metabolites are also found in some foods, including cow's milk [10]. Previous studies indicate that DM intoxication causes cellular injury and oxidative stress, which leads to lipid peroxidation and free radical production [11-14]. Recent reports have shown that acute and subchronic exposure to DM alters the antioxidant status and the histology of liver, brain, testes and lungs of animals [15-19] and human erythrocytes [20].

The kidney is an essential excretory organ which plays a dominant role in homeostasis by excreting metabolic waste products and excess necessary substances. It conserves necessary products depending on the needs of the body [21]. It is a prime target of several drugs, toxic xenobiotics or chemicals due to high rate of blood flow and presence of cellular transport systems that causes accumulation of these compounds within the nephron epithelial cells [22]. Metabolites of the drugs that are excreted from kidney may also cause cellular damage leading to kidney dysfunction. Increase in the levels of blood urea and creatinine is the principal diagnostic criteria of renal failure. Severe and progressive uremia may result in death [23]. The biochemical and histopathological evaluations are commonly used methods for detecting organ-specific effects related to chemical exposure [24].

Vitamin C (vitC) is a well-known antioxidant that protects the cellular compartment from water-soluble free radicals [25]. In addition, vitamin E (vitE) has been recognized to be the major lipid-soluble antioxidant that prevents free radicals from initiating peroxidative damage of tissues [26]. In addition, vitE inhibits free radical formation by scavenging lipid peroxyl

radicals, and is converted into α-tocopheroxyl radical as a consequence and may effectively minimize lipid peroxidation in biological systems [27-29]. Several experimental studies have shown that vitamins (C & E) could ameliorate pesticide toxicity [30-32]. Synergistic effect of antioxidants is most powerful in reducing storage and toxicity of reactive oxygen species [33, 34]. In fact, several studies demonstrated that the cellular antioxidant activity is reinforced by the presence of dietary antioxidants [35-37]. Accordingly, interest has recently grown in using antioxidants to prevent oxidative damage as a factor in the pathophysiology of various health disorders [38-42]. In this regard, studies on vitamin C and E are promising, mainly due to their antiradical activity, indicating that they could provide an important dietary source of antioxidants. So, the present study was undertaken to investigate some of the biochemical and histopathological alterations which might occur as a result of DM intoxication in the kidney of adult male guinea pigs. In addition, to study the protective potential of vitamins supplementation on DM induced nephrotoxicity.

2. MATERIALS AND METHODS

2.1 Chemicals

Dimethoate 40 EC was applied as a commercial emulsifiable concentrate formulation containing 40% active ingredient. Vitamin C (Shaphar, Shanghai pharmaceutical Co. Ltd., China) and vitamin E (Pharco Pharmaceutical, Alexandria, Egypt) were used for this study. Both the DM and vitE were reconstituted appropriately in olive oil for the final concentration immediately prior to use.

2.2 Animals and Treatment Schedule

Adult male guinea pigs (weighing 550–700g) were obtained from the animal house of Biology Department, Ibb University, Yemen and kept for 1 week on a commercial diet in environmentally controlled conditions with free access to diet and water ad libitum. Guinea pigs have been used because they, like humans, are incapable of synthesizing ascorbic acid; also, some metabolic characteristics in guinea pigs are similar to those in humans [43]. Animals were randomly divided into four groups with five animals each. The first group (G1) served as control and given saline (0.9% w/v) & olive oil. The second group (G2) received a combined dose of vitC and vitE at a dose of 200mg/kg b.w. /day orally. VitC and vitE were dissolved in water and olive oil, respectively. The third group (G3) orally received DM (7mg/kg b.w. per day; 1/50 of the LD50) dissolved in olive oil. The fourth group (G4) administrated with DM preceding by 30 min with vitC and vitE at the same previous dose of all. The regime schedule was selected according to previous studies [44,45]. All the previous administrations were repeated daily for 28 days. At the end of the 4th week (28 days) of treatment, the animals were sacrificed and dissected. Blood and tissue samples were taken for biochemical, and light microscope investigations. The appropriate animal care of Ibb University approved the protocol of the experiment.

2.3 Biochemical Evaluation

Serum urea concentration was determined using Biodiagnostic kits (Egypt) according to the method of Friedman and Young [46]. Serum creatinine concentration was determined using Biodiagnostic kits (Egypt) according to the method of Bartles et al. [47]. The levels of urea and creatinine were expressed as (mg/dl). Lipid peroxidation (LPO) was determined based on that of Ohkawa et al. [48]. A 10 (w/v) tissue homogenate from the kidney was required for

this assay (this homogenate contained 1%, v/v, dimethyl sulfoxide to prevent further oxidation). To 0.2ml Aliquots of tissue homogenate was added 0.2ml 8.1% (w/v) sodium dodecyl sulfate solution, 1.5ml 20% (v/v) acetic acid solution (pH 3.5) and 1.5ml 0.8% (w/v) thiobarbituric acid solution. The mixture was made up to 4.0 ml with distilled water and heated to 95ºC for 1h. The samples were cooled and centrifuged at 2000×g for 10 min and absorbance measured at 532nm. Results were expressed as nmolmalondialdehyde formation/mg protein. Catalase (CAT) activity was measured by the method of Aebi [49]. The reaction mixture was consisted of 0.5 ml phosphate buffer (50 mM, pH 7.0), 0.1ml of sample, 0.5ml of 30mM 1ml H_2O_2 and distilled water to make a total volume of 1.5ml. Change in absorbance was recorded at 240 nm. Catalase activity was calculated in terms of µmols H_2O_2 consumed /min/ mg protein. Glutathione-S-transferase (GST) activity was measured spectrophotometrically by the method of Habig et al. [50] using S-2, 4-dinitrophenyl glutathione (CDNB) as a substrate. The principle of the method is based on measurement of the conjugation of S-2, 4-dinitrophenyl glutathione (CDNB) with reduced glutathione. The formation of adduct of CDNB, S-2, 4-dinitrophenyl glutathione was monitored by measuring the net increase in absorbance at 340 nm against the blank. The activity of GST was expressed in terms of µmol/min/mg protein. The total protein content of kidney homogenate was determined by the method of Lowry et al. [51].

2.4 Histopathological Examination

Control and experimental animals were put under light ether anaesthesia, dissected as quickly as possible, and then the kidneys were removed. Small pieces were fixed in 10% neutral formalin for 24 hours, then washed by the running tap water, and stored in 70% ethyl alcohol, until further processing. Blocks of about 5 x 5mm size were dehydrated, cleared and embedded in paraffin wax. Paraffin sections of 5 microns thickness were cut using rotary microtome (Leica, Germany) and stained with haematoxylin and eosin.

2.5 Statistical Analysis

The quantitative values obtained were expressed as Mean ± S.D. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA). Differences with a *P*-value of <.05 were considered as statistically significant. Post hoc analysis of group differences was performed by LSD test. The treated groups were compared both with each other and with untreated control groups.

3. RESULTS AND DISCUSSION

At the end of the 4th week of our experiment, the control group was compared with all other groups. In addition, the DM-treated group was compared to the vitamins plus DM-treated group. There are no statistically significant differences when the vitamins-treated group was compared with the control group (Tables 1and 2). DM treatment (7mg/kg) for four weeks in guinea pigs caused a statistically significant increase (*P*<.O01) in the serum level of urea and creatinine when compared to control group (Table 1). Kidney is one of the targets organs of experimental animals attacked by OP compounds [52]. Urea and creatinine levels are kidney function parameters [53]. Pesticides can alter plasma urea and creatinine levels [54]. In fact, available data on the nephrotoxicity action of DM were limited for adult rats [55] and mice [56,52] and appeared to be lacking for adult guinea pigs. In the current study, oral administration of DM to adult male guinea pigs induced an increase in serum urea and creatinine which indicated a reduced urea and creatinine clearance efficiency. These

findings corroborated with previous investigations in adult rats treated by DM [57,55] and Malathion [58].

Levels of LPO were increased significantly (P<.001) in the kidney homogenates of DM-treated guinea pigs as compared to control group (Table 2). While the activity of CAT and GST were found to be inhibited significantly (P<.05) in DM treated group, as compared to the control group. Different mechanisms have been postulated to explain DM induced tissue injury, such as lipid peroxidation and interaction with membrane components resulting from free radicals' attack on biological structure [59]. Lipid peroxidation has been extensively used as a marker of oxidative stress. Our results were in consistence with previous studies which have shown that acute and subchronic exposure to DM generates lipid peroxidation and alters the antioxidant status of several tissues in rats [12,13,15,60]. In fact, the involvement of oxidative stress following exposure to OP has been reported [61,62,52].

On the other hand DM treatment in our study decreased CAT and GST activities. These antioxidants enzymes constitute the primary defense system that limits the toxicity associated with free radicals [36]. The decreased activity of CAT in DM administration could be due to the adaptive response to the generated free radicals indicating the failure of the total antioxidant defense mechanism to protect the tissues from mechanical damage caused by OP [63]. Also, the reduced activity of CAT in DM group may cause the accumulation of oxygen radical, H_2O_2 or their products of decomposition [64]. In addition, vitamins pre-treatment to DM-intoxicated guinea pigs resulted in a significant normalization (P<.O5) of the serum level of urea, creatinine, LPO, CAT, and GST as compared to the DM treated group alone (Table 2). Antioxidant vitamins have a number of biological activities, including immune stimulation and altering the metabolic activities of carcinogens. These vitamins can also prevent genetic changes by inhibiting the DNA damage induced by reactive oxygen metabolites [26]. One of the possible explanations involved in the detoxification following vitC and vitE treatment could be because these materials exert their protective influence by acting as antioxidants [65,66]. This finding supports Verma et al. [26], who reported that vitamins (C and E) efficiently inhibit *In vitro* lipid peroxidation in chlorpyrifos induced oxidative stress. Moreover, our light microscopic analyses revealed that the DM-treated animals which received vitamins co-administration did not exhibit the morphological changes seen in the kidneys of the DM-treated group. Thus, vitC and vitE could ameliorate the tissue damage induced by DM intoxication.

Table 1. Serum urea and creatinine in control and different treated male guinea pigs

Parameters	Control	Vitamins C & E	DM	Vitamins + DM
Urea (mg/dl)	55.0±9.17[a]	57.60±7.96[a]	81.80±6.34[b]	65.80±12.85[c]
Creatinine (mg/dl)	0.49±0.10 [a]	0.53±0.14 [a]	1.21±0.12 [b]	0.87±0.17 [c]

Values are expressed as Mean ± SD; n= 5 for each treatment group. Means in the same row assigned with the same letter show insignificant differences; [b]Significantly different from controls. [c] Significantly different from DM-treated animals

There are several reports supported the role of antioxidant in attenuating the histopathology of some pesticides and toxins in experimental animals. For example, selenium and vitamin E ameliorated the toxic effects of DM in lung tissue [67]. Also, ascorbic acid supplementation prevents the testicular damage induced by DM intoxication [68]. In the present study, kidneys of the control and vitamin-treated group showed a normal structure (Figs. 1A, 1B). Histopathological changes were observed in the kidneys of DM-treated group and vitamins plus DM treated group compared to control group (Figs. 1C-E). After 4 weeks of DM administration, many histopathological changes were noted like cytoplasmic vacuolization, cellular infiltration, vascular congestion, tubular degeneration, glomerular atrophy and

regions of necrosis (Figs. 1C, D) when compared with those of controls (Fig. 1A). The histopathological changes were also observed in the kidney sections of vitamin plus DM treated group but were less pronounced (Fig. 1E).

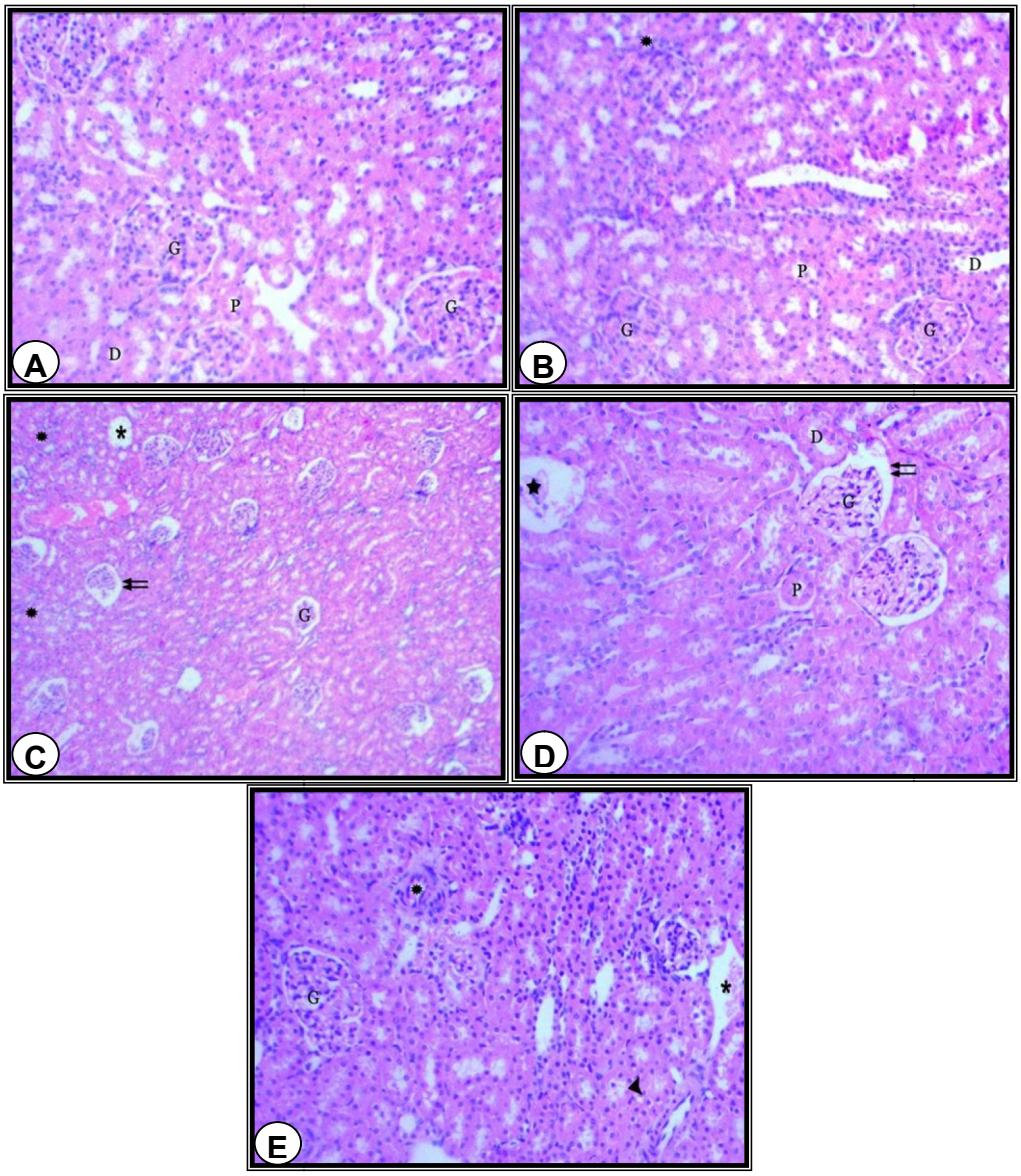

Fig. 1. (A) Kidney section of control guinea pig, X200. (B) Kidney section of vitC & vitE - treated guinea pig, X200. (C) Glomerular atrophy (G) and vascular dilatation (✱), cellular infiltration (✹) in kidney tissue 4 weeks after DM treatment to guinea pig, X140. (D) Enlarged portion in kidney 4 weeks after DM treatment to guinea pig showed glomular atrophy (G), break away from basal membrane (⇉), and necrosis (★) X200. (E) Tubular degeneration (◀), vascular dialation (✱), and cellular infiltration in kidney 4 weeks after vitamins +DM treatment to guinea pigs, X200. P, Proximal tubules; D, distal tubules; G, Glomerulus

Organophosphate insecticides are known to induce various histopathological changes in the kidney tissues of experimental animals [44,64,69,70]. Moreover, we noticed that these changes were also appeared in vitamins plus DM-treated group but with little severity. In addition, the alterations in biochemical parameters were well correlated with the histopathological changes. In supporting to our finding, DM induced similar histopathological changes in kidney tissues of female rats and their pups [57]. In addition, our results confirmed previous findings of Sulak et al. [70] and Kalender et al. [71] who had found degenerative changes in kidney of adult rats exposed to methidathion and methyl parathion. Moreover severe interstitial mononuclear cells' infiltration [72], hyperplasia and hypertrophy of tubular cells [71] had been also observed.

Table 2. LPO, activities of CAT and GST in the kidney of control and different treated male guinea pigs

Parameters	Control	Vitamins C & E	DM	Vitamins + DM
LPO (nmol/mg protein)	1.52±0.33[a]	1.68±0.27[a]	2.95±0.65[b]	1.81±0.27[c]
CAT (µmol/min/mg protein)	4.40±1.18[a]	5.01±1.42[a]	1.95±0.83[b]	4.41±1.01[c]
GST (µmol/min/mg protein)	26.50±3.03[a]	30.36±7.62[a]	12.07±2.69[b]	33.25±8.11[c]

Values are expressed as Mean ± SD; n= 5 for each treatment group. Means in the same row assigned with the same letter show insignificant differences. [b] Significantly different from controls. [c] Significantly different from DM-treated animals

4. CONCLUSION

This study may constitute the first attempt to evaluate the effects of vitamins (C & E) on DM-induced nephrotoxicity in adult guinea pigs. The results of the present study illustrated that administration of vitamins is capable of reversing the oxidative toxic effects of DM. These data suggest that vitamins, by preventing pesticides toxicity, may enhance the selectivity of these vitamins in the patients who occupationally exposed to DM insecticide.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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