Effect of Vitamin E Supplementation on Biochemical Parameters in Pesticides Sprayers of Grape Gardens of Western Maharashtra (India)

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Abstract The aim of this study was to see the biochemical effects of pesticides on sprayers of grape gardens before and after 15 days of vitamin E supplementations in Western Maharashtra (India), who were occupationally exposed to various pesticides over a long period of time (about 5 to 15 years). Blood samples were collected from all study group subjects for biochemical parameters assays before and after 15 days of vitamin E supplementation. Sprayers of grape gardens were given 400 mg of vitamin E tablet/day for 15 days. After 15 days of vitamin E supplementation to sprayers of grape gardens, we observed significantly decreased aspartate transaminase (10.88 %, $P < 0.05$, $r = 0.88$), alanine transaminase (25.92 %, $P < 0.01$, $r = 0.46$) and total proteins (3.32 %, $P < 0.01$, $r = 0.33$), whereas, no statistically significant change was found in serum acetyl cholinesterase, C-reactive proteins, albumin (ALB), globulins and ALB/globulin ratio as compared to before vitamin E supplementation. Sprayers of grape gardens, who received vitamin E supplementation, showed significantly decreased serum lipid peroxide (LP) (18.75 %, $P < 0.001$, $r = 0.63$) and significantly increased RBC-superoxide dismutase (SOD) (12.88 %, $P < 0.001$, $r = 0.85$), RBC-Catalase (CAT) (24.49 %, $P < 0.001$, $r = 0.70$), plasma ceruloplasmin (CP) (4.6 %, $P < 0.01$, $r = 0.80$), serum zinc (4.57 %, $P < 0.01$, $r = 0.83$) and serum copper (4.37 %, $P < 0.01$, $r = 0.79$) as compared to

values before vitamin E supplementation. These results showed that vitamin E supplementation has ameliorating effects on these transaminase enzymes, suggesting that it may have a protective effect on liver, from pesticides induced damage. In this study vitamin E supplementation might have decreased LP levels by breaking chain reaction of lipid peroxidation. Present results indicate that vitamin E plays a crucial role in restoring the antioxidant enzymes such as SOD, CAT and CP, in population exposed to pesticides. This helps to enhance its antioxidant ability. Therefore, it is suggested that farmers, pesticide applicators, workers in the pesticide industry and other pesticide users, who come in regular contact with pesticides, may be benefited by supplementation with vitamin E.

Keywords Acetylcholinesterase · C-reactive proteins · Aspartate transaminase · Alanine transaminase · Lipid peroxidation · Superoxide dismutase · Catalase · Ceruloplasmin · Glutathione-S-transferase

Introduction

Pesticides are among the most widely used chemicals in the world which are very dangerous to human health. Currently over 2.5 million tons of such chemicals, worth over US\$30 billion, are applied to crops in every country in the world [1]. In a developing country like India, with agriculture based economy, there is an increasing trend of cash-crop cultivation. In the horticulture sector, the land used for cash crop like grapes is increasing, particularly in Maharashtra state. Hence there is increased use of the pesticides in an attempt to increase the yield and reduce post harvest losses. Environmental pollution and poisoning due to wide spread use of pesticides during grape cultivation may be an

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important factor that can disturb the socio economical status of uneducated farm workers in rural areas [2].

Pesticides are ubiquitous contaminants of our environment. They have been found in air, soil, water as well as human and animal tissues all over the world. Pesticides are chemical substances used to kill animals, insects, plant and fungal pests in agricultural, domestic and institutional settings. The principle classes of compounds that have been used as insecticides are organochlorines, organophosphorous, carbamate and pyrethroids compounds, and various inorganic compounds.

Pesticides uptake occurs mainly through the skin and eyes, by inhalation or by ingestion. The fat-soluble pesticides and to some extent, water-soluble pesticides are absorbed through intact skin. Sores and abrasions may be facilitating the uptake through the skin. The vapours of pesticides or aerosol droplets smaller than 5 μm in diameter are absorbed effectively through the lungs. Larger inhaled particles or droplets may be swallowed after being cleared from the airways. Ingestion can occur from the consumption of contaminated food or by using contaminated utensils [3]. Occupational exposures occur in the mixing and loading of equipment as well as in the spraying and application of insecticides. There are several factors which affect the levels of exposure while mixing and handling during the agricultural application of pesticides [4]. Other factors i.e. wind, equipment used, duration of exposure and individual protection also decide the effect of pesticide exposure [5].

Signs and symptoms associated with mild exposures to organophosphate and carbamate insecticides include headache, fatigue, dizziness, loss of appetite with nausea, stomach cramps, diarrhea, blurred vision, contracted pupils of the eye, excessive sweating, salivation, bradycardia etc.

Pesticides affect number of enzymes and physiological systems i.e. reproduction, nervous, immune, endocrine, blood coagulation, hematology, cardiovascular, respiration, metabolisms and fluid and electrolyte balance. They have carcinogenic and mutagenic potential also. It affects several organs of human beings, but liver is most susceptible [6, 7]. The increased formation of reactive oxygen and nitrogen species, resulting to increased lipid peroxidation in several tissues, mainly brain, skeletal muscle, RBC, etc. and depletion of antioxidant status, were reported in several studies of various pesticide exposed population [4, 5].

Therefore our aim of this study was to assess the effects of pesticide exposure on oxidative stress (lipid peroxide, LP), antioxidant status such as RBC-superoxide dismutase (SOD), catalase (CAT), plasma ceruloplasmin (CP), glutathione-S-transferase (GST), acetyl cholinesterase (AChE), C-reactive proteins (CRP), total proteins (TP), albumin (ALB), globulins, A/G ratio and trace elements i.e. zinc (Zn) and copper (Cu) in sprayers of grape gardens

before and after oral antioxidant supplementation of vitamin E.

Materials and Methods

Thirty subjects with occupational exposure to pesticides i.e. sprayers of grape gardens were taken for this study. All the study group subjects were of the age group between 20 to 45 years from Tasgaon taluka of Sangli district, in Western Maharashtra, India. All the study group subjects were given vitamin E supplementation (400 mg/day) for 15 days. Sprayers of grape gardens and grape growers were informed about the study objectives and health hazards of pesticides exposure prior to data and biological specimen collection. Written consent was obtained from all sprayers of grape gardens. Demographic, occupational and clinical data were collected by using questionnaire and interviews. Most of the sprayers of grape gardens had major complaints of lacrimation, nausea, salivation, sniffing, headache, breathlessness, itching and vomiting. All the subjects of the study groups belonged to agricultural family with similar socioeconomic status. None of the subjects had a past history of major illness. Dietary intake and food habits of all subjects were normal which was confirmed periodically by checking their tiffins during their lunch. It was also verified that they had their routine breakfast and dinner. The subjects, who were on drugs for minor illnesses were excluded from this study. Non-smokers, non-alcoholic healthy males, occupationally exposed to various pesticides i.e. sprayers of grape gardens for more than 5 to 15 years duration and after October pruning with daily exposure of 4 to 5 h for 3 months were selected for this study. All these study group subjects were mainly exposed to Lannate (Methomyl), Endosulphan, Novan, Demecron, Basathrin etc. pesticides commonly used in all grape gardens. The entire experimental protocol was approved by the institutional ethical committee and utmost care was taken during the experimental procedure according to the Helsinki Declaration of 1964 [8]. Blood from sprayers of grape gardens was collected by venipuncture into evacuated tubes containing heparin solution as anticoagulant for biochemical parameters assay before and after 15 days of vitamin E supplementation.

From all subjects of study group, serum AChE, CRP, aspartate transaminase (AST), alanine transaminase (ALT), TP, ALB, GLB, A/G ratio, LP and antioxidants status parameters i.e. RBC-SOD, CAT, CP, GST, serum Zn and serum Cu were measured before and after vitamin E supplementation using standard methods.

Serum AChE was measured by Knedel et al. [9] by using Accucare kit method. The butyrylthiocholine is hydrolysed by serum cholinesterase to produce thiocholine

in the presence of potassium hexacyanoferrate (III). The absorbance decrease is proportional to the cholinesterase activity of the sample [9].

Serum CRP was measured by Andersen and McCarthy [10] and Lothar Thomas [11] TURBILYTE-CRP™. It is a turbidimetric immunoassay for the determination of CRP in human serum and based on the principal of agglutination reaction. The serum sample is mixed with activation buffer (R1), TURBILYTE-CRP™ latex reagent (R2) and allowed to react. Presence of CRP in the serum sample results in the formation of an insoluble complex producing turbidity which was measured at 546 nm wavelength. The increase in turbidity corresponds to the concentration of CRP in the serum specimen.

The liver function tests were measured by using a fully automated biochemistry analyzer (Eurolyser) on the same day of sample collection. The AST and ALT were measured by the UV-kinetic method using reagents from M/S Accurex Biomedical Ltd. The conversion of NADH to NAD in both transaminase (AST, ALT) reactions was measured at 340 nm as the rate of decrease in absorbance [12].

Serum total proteins were measured by the Biuret method [13] using an M/S Accurex Biomedical Kit. Serum proteins react with cupric ion in alkaline pH to produce a colored complex. The intensity of the color complex was measured at 546 nm. Serum ALB was measured by the BCG method using reagents from M/S Beacon Ltd. Serum ALB binds with 3,3',5,5'-tetra bromocresol green in acidic medium at pH 4.2 and the blue-green colored complex formed was measured at 600 nm. Serum globulins and the A/G ratio were calculated by using serum total proteins and ALB values [14].

Lipid peroxidation was measured spectrophotometrically by method of Kei Satoh [15]. Serum proteins were precipitated by trichloroacetic acid (TCA) and the mixture was heated for 30 min with thiobarbituric acid in 2 M sodium sulfate in a boiling water bath. The resulting chromogen is extracted with *n*-butyl alcohol and the absorbance of the organic phase was determined at the wavelength of 530 nm. The values were expressed in terms of malondialdehyde (MDA) nmol mL⁻¹ using 1,1,3,3-tetraethoxy propane as the standard [15].

The activity of RBC-SOD was measured by the method of Marklund and Marklund [16]. Superoxide anion is involved in the auto-oxidation of pyrogallol at alkaline pH 8.5 and is inhibited by SOD which can be determined as an increase in absorbance per 2 min at 420 nm. The SOD activity was measured as unit mL⁻¹ hemolysate. One unit of SOD is defined as the amount of enzyme required to cause 50 % inhibition of pyrogallol auto-oxidation [16].

RBC-Catalase was measured by the method of Aebi [17]. Heparinized blood was centrifuged and plasma was

removed and the erythrocytes were washed 2–3 times with 0.9 % NaCl and then lysed in ten volumes of cold deionized water. The whole mixture was centrifuged for 10 min at 3,000 rpm. The cell debris was removed and the clear hemolysate was diluted 500 times with phosphate buffer (60 mM) pH 7.4. CAT decomposes H₂O₂ to form water and molecular oxygen. In the UV range, H₂O₂ shows a continual increase in the absorption with decreasing wavelength. At 240 nm H₂O₂ absorbs maximum light. When H₂O₂ is decomposed by CAT then the absorbance decreases. The decreased absorbance was measured at 240 nm for every 15 s interval up to 1 min and the difference in absorbance (ΔA at 240 nm) per unit time is a measure of the CAT activity. The unit of CAT activity was expressed as mM of H₂O₂ decomposed/mg Hb min⁻¹ [17].

Plasma ceruloplasmin was measured by the method of Herbert and Ravin [18]. CP oxidizes *p*-phenyl-enediamine in presence of oxygen to form a purple-colored oxidized product. The ceruloplasmin concentration was determined from the rate of oxidation of *p*-phenyl-enediamine at 37 °C at pH 6.0 which has an absorption peak at 530 nm [18].

Serum GST was measured by using Habig et al. [19] method. GST activity was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample [19].

Serum Zn and Cu were measured using a Perkin Elmer model 303 graphite furnace atomic absorption spectrophotometer which was connected to Hitachi 165 recorder; values were shown in $\mu\text{g dL}^{-1}$ [20, 21].

Statistical comparisons between results of sprayers of grape gardens (before and after vitamin E supplementation) were made by student *t* test. Pearson's correlation equation was also done to evaluate proper correlation between biochemical parameters in respect of sprayers of grape gardens before and after vitamin E supplementation.

Results and Discussion

The α -tocopherol (vitamin E) acts as a chain-breaking antioxidant for lipids in biological membranes and is used in medicine in the treatment of cataract, atherosclerosis, cancer and peripheral neuropathy, but no one has carried out studies indicating its effect on sprayers of grape gardens elsewhere. Few reports assessing immunological and hematological changes in pesticide sprayers who are occupationally exposed to individual pesticides are available [22]. No detailed report is available on lipid peroxidation, antioxidant status and biochemical parameters of liver functions in respect of sprayers of grape gardens who are occupationally exposed to pesticides. Similarly the role

Table 1 Depicts mean values and correlation coefficient (*r*) of serum acetyl cholinesterase, C-reactive proteins, liver functions tests of sprayers of grape gardens before and after vitamin E (400 mg/tab/day for 15 days) supplementation

Parameters	Vitamin E		Correlation coefficient (<i>r</i>)
	Before (<i>N</i> = 30)	After (<i>N</i> = 30)	
AChE [U/L]	5010 ± 1000 (3258–6602)	5114 ± 955* (3391–6692)	0.795
CRP [mg/dl]	0.156 ± 0.19 (0.045–0.89)	0.16 ± 0.18* (0.023–0.789)	0.820
AST [U/L]	28.65 ± 14.69 (14–78)	25.30 ± 9.15* (16–54)	0.880
ALT [U/L]	35.10 ± 16.5 (18–88)	26 ± 9.21** (12–44)	0.460
TP [gm/dl]	7.53 ± 0.30 (6.8–7.8)	7.28 ± 0.21** (6.9–7.7)	0.337
ALB [gm/dl]	4.20 ± 0.20 (3.8–4.6)	4.25 ± 0.19* (3.9–4.6)	0.180
GLB [gm/dl]	3.07 ± 0.31 (2.3–3.6)	3.04 ± 0.29* (2.10–3.5)	0.379
A/G ratio	1.37 ± 0.16 (1.05–1.78)	1.40 ± 0.12* (1.14–1.76)	0.235

Figures indicate mean ± SD values and those in parenthesis are range of values

Non significant with respect to before vitamin E supplementation of sprayers of grape gardens

Acetyl cholinesterase (*AChE*), C reactive proteins (*CRP*), aspartate transaminase (*AST*), alanine transaminase (*ALT*), total proteins (*TP*), albumin (*ALB*), globulins (*GLB*)

** *P* < 0.01, * *P* < 0.05

of α -tocopherol supplementation in these sprayers also has not been studied.

Burton et al. [23] have reported that vitamin E is the only lipid soluble antioxidant present in plasma and erythrocytes membranes and have indicated that this vitamin is the only radical chain breaking substance. Vitamin E ameliorates the effect of a large number of insults to experimental animals which include high oxygen tension, ozone, nitrogen dioxide, mercury, lead, ethanol, carbon tetrachloride, paraquat, nitrosamine [24] and drugs such as acetaminophen, digitalis and adriamycin [25].

In sprayers of grape gardens who were given vitamin E supplementation for 15 days we found significantly decreased levels of serum AST (10.88 %, *P* < 0.05, *r* = 0.88), serum ALT (25.92 %, *P* < 0.01, *r* = 0.46) and TP (3.32 %, *P* < 0.01, *r* = 0.33). Whereas no statistically significant change was found in AChE, CRP, ALB, globulins, and A/G ratio when compared with levels before vitamin E supplementation (Table 1; Fig. 1).

In our earlier study, increased AST and ALT activities were observed in sprayers of grape gardens as compared to control subjects [26]. After 15 days of vitamin E supplementation to sprayers of grape gardens we found that the activities of AST and ALT were decreased significantly. A good correlation coefficient (*r*) of AST and ALT before and after vitamin E supplementation was also observed which indicates that in majority of sprayers these transaminase enzyme activities were reduced after vitamin E supplementation. The significant elevation in the activity of these transaminase enzymes in this study indicates the damage to liver by various pesticides.

Fig. 1 Percentage change of mean values of serum acetyl cholinesterase, C-reactive proteins, liver functions tests of sprayers of grape gardens after vitamin E (400 mg/tab/day for 15 days) supplementation with respect to before vitamin E supplementation (Ref. Table 1). Acetyl cholinesterase (*ACh*), C reactive proteins (*CRP*), aspartate transaminase (*AST*), alanine transaminase (*ALT*), total proteins (*TP*), albumin (*ALB*), globulins (*GLB*)

Several studies have reported that the vitamin E [27–30] and selenium-vitamin E combination [31, 32] supplementation protected the liver against damage. The decreased AST and ALT after vitamin E supplementation in this study indicates ameliorating effects. In addition, vitamin E and selenite compound are complementary to each other in preventing hepatic necrosis or muscular dystrophies [33, 34]. Vitamin E is also used to prevent oxidative damage by interrupting the propagation of the oxidation of polyunsaturated fatty acids. Some investigators reported that administering vitamin E may be useful in controlling the hepatotoxic effects of insecticides and chemicals [35, 36].

Table 2 Mean values and correlation coefficient (*r*) of lipid peroxide, antioxidant enzymes and trace elements of sprayers of grape gardens before and after vitamin E (400 mg/tab/day) for 15 days supplementation

Parameters	Vitamin E		Correlation coefficient (<i>r</i>)
	Before (<i>N</i> = 20)	After (<i>N</i> = 20)	
LP [nmol/ml]	3.36 ± 0.76 (2.81–6.17)	2.73 ± 0.52*** (2.01–3.66)	0.631
SOD ^a	10.09 ± 1.65 (8.9–16.23)	11.39 ± 2.19*** (7.4–18.30)	0.851
CAT ^b	9.47 ± 4.30 (4.23–21.13)	11.79 ± 5.32*** (4.22–29.58)	0.705
CP [mg/dl]	70.34 ± 16.65 (33.6–93.54)	73.58 ± 18** (35.46–99.6)	0.801
GST ^c	0.090 ± 0.051 (0.023–0.215)	0.076 ± 0.044* (0.011–0.22)	0.135
Serum Zn [μg/dl]	83.77 ± 10.9 (70–115)	87.6 ± 11.4** (74–118)	0.830
Serum Cu [μg/dl]	81.60 ± 13.6 (55–100)	85.17 ± 12.4** (58–105)	0.790

Figures indicate mean ± SD values and those in parenthesis are range of values

Non significant with respect to the before vitamin supplementation of sprayers of grape gardens

Lipid peroxide (LP), RBC-superoxide dismutase (SOD), RBC-catalase (CAT), plasma ceruloplasmin (CP), glutathione S-transferase (GST)

^a Unit/ml of hemolysate; ^b mM H₂O₂ decom/mg Hb/min; ^c μmol of conjugate form/min/mg of protein

*** *P* < 0.001, ** *P* < 0.01, * *P* < 0.05

Generally vitamin E is required for the optimum absorption of amino acids from the intestine [33, 34]. But slightly decreased total proteins levels (3.32 %, *P* < 0.01, *r* = 0.33) in sprayers of grape gardens after vitamin E supplementation as compared to before the supplementation of vitamin E is not very clear.

We observed significantly decreased levels of LP (18.75 %, *P* < 0.001, *r* = 0.63) and increased RBC-SOD (12.88 %, *P* < 0.001, *r* = 0.85), RBC-CAT (24.49 %, *P* < 0.001, *r* = 0.70), CP (4.6 %, *P* < 0.01, *r* = 0.80), Zn (4.57 %, *P* < 0.01, *r* = 0.83), Cu (4.37 %, *P* < 0.01, *r* = 0.79) in sprayers of grape gardens after the supplementation of vitamin E as compared to before vitamin E supplementation (Table 2; Fig. 2).

Vitamin E is involved in removal of free radicals preventing their peroxidative effects on unsaturated lipids of membranes and thus helps to maintain the integrity of cell membranes. α-tocopherol reacts with the LP radicals formed by peroxidation of polyunsaturated fatty acids before they can establish a chain reaction acting as free radical trapping anti-oxidant. The tocopheroxy-free radical (Toc.O•) product formed in the process is relatively unreactive and ultimately forms non-radical compounds. Usually the tocopheroxyl radical is reduced back to α-tocopherol again by reacting with vitamin C or reduced glutathione GSH [33, 34]. Hence in this study vitamin E supplementation may be producing decreased LP levels by breaking chain reaction of lipid peroxidation.

We observed significantly increased RBC-SOD (12.88 %), RBC-CAT (24.49 %) and CP (4.6 %), in sprayers of grape gardens after 15 days of vitamin E supplementation as compared to levels before vitamin E supplementation (Table 2; Fig. 2).

Fig. 2 Percentage change mean values of lipid peroxide, antioxidants enzymes and trace elements of sprayers of grape gardens after vitamin E (400 mg/tab/day for 15 days) supplementation with respect to before vitamins supplementation (Ref. Table 2). Lipid peroxide (LP), RBC-superoxide dismutase (SOD), RBC-catalase (CAT), plasma ceruloplasmin (CP), glutathione-S-transferase (GST)

The human body has several mechanisms to counteract the damage caused by free radicals. The basic and the most prominent defense mechanism of the human body are antioxidant agents. The term antioxidant is defined as any substance that delays or inhibits oxidative damage to a target molecule. These molecules are stable enough to neutralize free radicals by donating electrons. Today many compounds have found to have antioxidant activity. In the human body they can be categorized in two systems. The main system of defense against damage from free radicals is the enzymatic system that opposes oxidation [37]. The body maintains pools of the antioxidant vitamins such as vitamin E, vitamin C and β-carotene, the vitamin A precursor. This first defense system tries to handle all free

radicals. If the oxidative stress is far greater than the capacity of the system, the second line of defense (vitamins) may come into play. Vitamins scavenge and quench free radicals but are oxidized and inactivated in the process. Each of these antioxidant nutrients has specific activities and they often work synergistically to enhance the overall antioxidant capacity of the body [38].

Previous studies have shown that chlorpyrifos-ethyl (OP pesticide) causes an increase in LP and decrease in the activity of GSH Px, SOD, and CAT, but these effects are reversed by pre-treatment with vitamin C and E [39, 40]. Therefore from past reports and present results it is indicated that vitamin E plays a crucial role in restoring the antioxidant enzymes such as SOD, CAT and CP in population exposed to pesticides.

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