The aim of this study was to investigate the biochemical effects of pesticides on sprayers of vineyards in Western Maharashtra (India), before and after 15 days of taking vitamin C supplements, 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 C supplements. The study clearly showed that giving vitamin C supplements protects liver damage in vineyard crop sprayers, which results in decreased aspartate transaminase and alanine transaminase activity. In addition to its antioxidant activity, vitamin C is known to perform other actions that enhance its protective effect in OP (organophosphate)-induced toxicity.

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 benefit from pretreatment with vitamin C.

Key words: Acetylcholinesterase, C-reactive protein, Aspartate transaminase, Alanine transaminase, Lipid peroxidation, Superoxide dismutase, Catalase, Ceruloplasmin, Glutathione S-transferase, Zinc, Copper.

Introduction

Grape cultivation is increasing, mainly in Western Maharashtra (India). Nowadays, grape growers are using more pesticides to increase the yield and reduce the post-harvest losses. The environmental pollution and poisoning caused by widespread use of pesticides during grape cultivation may be important factors affecting the socio-economic status of uneducated farm workers in rural areas [Dave, 1998].

Pesticides are ubiquitous contaminants of the environment and are found in air, soil, water, human and animal tissue samples from all over the world. The common organophosphorous and carbamate pesticides used in vineyards are Basathrin 25 EC (Cypermethrin 25% EC), Nuvan (Dichlorovas 76% EC), Nuvacron (Monocrotopho 36% EC), Dimethoate 30% EC (CHAMP 30 EC), Phoshamidon 85% SL (Dimecron), Kilex Endosulfan 35% EC, Carbaryl, Cypermethrin 25% EC (JAWAA), Monocrotophos 36% SL, and Methomyl.

The principal classes of compounds used as insecticides are organochlorines, organophosphorous, carbamates and pyrethroid 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, the water-soluble pesticides are absorbed through intact skin. Sores and abrasions may facilitate uptake through the skin. The fumes from pesticides or aerosol droplets smaller than 5µm in diameters are absorbed effectively through the lungs. Larger inhaled particles or droplets may be swallowed after being cleared from the airways [WHO, 1990].

Occupational exposure occurs in the mixing, and loading of equipment and in the spraying and application of the insecticides. There are several factors affecting the levels of exposures that occur while mixing and handling during the agricultural application of pesticides [Wolfe et al., 1967].

Other factors are wind, equipment used, duration of exposure, and individual protection [MacCollom et al., 1986]. Absorption resulting from dermal exposure is the most important route of uptake for exposed workers. Acute toxic effects are easily recognised, whereas the effects resulting from long-term exposure to low doses are often difficult to distinguish. In particular, the effects of a regular intake of pesticide residues in food are hard to detect and quantify [WHO, 1990].

Signs and symptoms associated with mild exposure to organophosphate and carbamate insecticides include: headache, fatigue, dizziness, loss of appetite with nausea, stomach cramps, diarrhoea, blurred vision associated with excessive watering of the eyes, contracted pupils, excessive sweating and salivation, slowed heartbeat (often fewer than 50 per minute), and rippling of surface muscles just under the skin [WHO, 1990; Al-Saleh, 1994].

The adverse effect from exposure to pesticides depends on the dose, the route of exposure, how easily the pesticide is absorbed and persistence in the body. The toxic effect also depends on the health status of the individual. Malnutrition and dehydration are likely to increase sensitivity to pesticides. Pesticides have been known to affect a number of enzymes and physiological systems, which results in a wide variety of changes in humans.

Pesticides have been shown to affect mammalian reproduction, nervous, immune, and blood coagulation systems and they have carcinogenic and mutagenic potential. Exposure affects several organs in humans, but
the liver is most susceptible [WHO, 1992; WHO, 1993]. Disorders of the cardiovascular system, nervous system, sensory organs, respiratory system, and reduced lung function have been reported after exposure to pesticides. Skin disorders including dermatitis, headache and nausea have also been reported [IARC, 1991].

Genotoxic effects are considered to be the most serious possible side effects of agricultural chemicals. If the chemical reacts with nuclear DNA, it is usually mutagenic and carcinogenic to the exposed organisms. The effects include inheritable genetic diseases, carcinogenesis, reproductive dysfunction and birth defects [Wagida, 1997].

Accumulation of acetylcholine in the CNS (central nervous system) is believed to be responsible for the tension, anxiety, restlessness, insomnia, headache, emotional instability, neurosis, excessive dreaming, nightmares, apathy and confusion described after organophosphorus (OP) pesticide poisoning. Slurred speech, tremors, generalised weakness, ataxia, convulsions and coma are the other CNS effects [Ecobichon, 1996].

Metabolic disturbances, fluid and electrolyte imbalance are also reported in several OP and carbamate-exposed populations. The increased formation of reactive oxygen and nitrogen species results in an increase in lipid peroxidation in the brain, musculo-skeletal system, RBC, etc. and depletes antioxidant status reported in several studies of various pesticide-exposed populations [Dave, 1998; FAO Rome, 1986; WHO, 1990; Wolfe and MacCollom et al., 1986; Al-Saleh, 1994].

The pesticides may irritate lung macrophages, encouraging them to generate superoxide radicals and deplete antioxidants status. The biochemical effect produced by certain pesticides can be enzyme induction or inhibition. Several pesticides inhibit cholinesterase, altered liver and kidney functions, decreased haemoglobin, impaired oxidative stress, antioxidants imbalance and altered drug metabolism of liver enzymes has been reported among pesticide-exposed workers [WHO, 1992 and 1993; Patil et al., 2003].

In an earlier study, we found altered haematological parameters, liver and kidney functions, along with impaired mixed-function oxidase systems, oxidative stress and antioxidants imbalance [Patil et al., 2003 and 2008]. Ascorbic acid (Vitamin C) is an important antioxidant that significantly decreases the adverse effect of reactive oxygen and oxides of nitrogen that can cause oxidative damage to macromolecules such as lipids, DNA and proteins. Vitamin C plays a crucial role in wound healing and reducing inflammation, and may partially prevent certain types of hepatic cellular damage.

Ascorbic acid also regenerates other small molecule antioxidants such as α-tocopherol, glutathione, urate and β-carotene from their respective radical species [Frei et al., 1990; Halliwell, 1996]. It also enhances protein biosynthesis. The chief source of vitamin C is citrus fruits, which are predominantly available in the field. Therefore, this study was undertaken to investigate the biochemical effects of pesticides on vineyard crop sprayers before and after 15 days of giving vitamin C supplements.

Materials and methods

This study comprises 30 subjects with occupational pesticides exposure, i.e. sprayers of vineyards. All the study-group subjects had ages in the range of 20 to 45 years and came from Tasgaon taluka (an administrative unit of a district), Sangli district, (Western Maharashtra) India. For all study-group subjects, a 500mg vitamin C tablet/day for 15 days was given to each participant. Both growers and sprayers were informed of the study objectives and health hazards of pesticides exposure prior to data and biological specimen collection. Written consent was obtained from all sprayers.

Demographic, occupational and clinical data were collected by using questionnaire and interview. Most of the crop sprayers had major problems of watering eyes, nausea, salivation, sniffing, headache, breathlessness, itching and vomiting. All the subjects of the study groups belong to agricultural families with similar socio-economic 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 what they ate at lunchtime. It was also verified that they had their routine breakfast and dinner.

Any 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. vineyard sprayers for between 5-15 years duration of exposure - were selected for this study. 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 [Helsinki, 1964]. Blood samples were taken from crop-sprayers’ veins into tubes containing heparin solution as anticoagulant for
Biochemical effects of pesticides on crop sprayers in Western Maharashtra (India) vineyards before and after fifteen days of taking vitamin C supplements

biochemical parameters assay before and after 15 days of vitamin C supplementation

From all subjects within the study group, serum acetyl cholinesterase (ACHE), C reactive proteins (CRP), aspartate transaminase (AST), alanine transaminase (ALT), total proteins (TP), albumin (ALB), globulin (GLB), A/G ratio, lipid peroxide, and antioxidants status parameters, i.e. RBC-superoxide dismutase (SOD), RBC-Catalase(CAT), plasma ceruloplasmin (CP), glutathione S-transferase (GST), serum zinc (Zn) and serum copper (Cu) were measured before and after giving vitamin C supplement to crop sprayers, using standard methods.

Serum Acetyl Cholinesterase was measured by the Knedel et al. [1989] Accuca re kit method. The Butyrylthiocoline is hydrolysed by serum cholinesterase to produce thiocoline in the presence of potassium hexacyanoferratere (III). The absorbance decrease is proportional to the cholinesterase activity of the sample.

Serum C-Reactive Proteins was measured by the Anderson and McCarthy [1950], Lothar Thomas [1998] method. TURBILYTE- CRP™ is a turbidimetric immunoassay for the determination of C-reactive protein 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 a turbidity, which is 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 analyser (Eurolleyer) on the same day of sample collection. The SGOT (AST) and SGPT (ALT) were measured by the UV-kinetic method [Committee on Enzymes of the Scandinavian Society, 1974] using reagents from M/S Accurex Biomedical Ltd. The conversion of NADH to NAD in both transaminase reactions was measured at 340nm, as the rate of decrease in absorbance.

Serum total proteins were measured by the Biuret method [Henry et al., 1974] using an M/S Accurex Biomedical Kit. Serum proteins react with cupric ion in alkaline pH to produce a coloured complex; the intensity of the colour complex was measured at 546nm and directly proportional to the protein concentration in the specimen. Serum albumin was measured by the BCG method [Daumas et al., 1971] using reagents from M/S Beacon Ltd. Serum albumin binds with 3,3′,5,5′-tetramethylbenzidine (BCG) in acidic medium at pH 4.2, and the blue-green coloured complex formed is measured at 600nm. Serum globulins and the A/G ratio were calculated by using serum total proteins and albumin values.

Lipid peroxidation was measured spectrophotometrically by the Satoh [1978] method. Serum proteins were precipitated by trichloroacetic acid (TCA) and the mixture was heated for 30 min with thiobisulphuric acid in 2M sodium sulphate, in a boiling water bath. The resulting chromogen was extracted with n-butyl alcohol and the absorbance of the organic phase was determined at a wavelength of 530nm. The values were expressed in terms of malondialdehyde (MDA) nmol mL⁻¹ using 1, 1, 3, 3, tetraethoxy propane as the standard.

The activity of erythrocyte superoxide dismutase (SOD) was measured by the Marklund and Marklund [1988] method. 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 two minutes at 420nm. The SOD activity was measured as units mL⁻¹ hemolyase. One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation.

Erythrocyte catalase was measured by the Aebl [1983] method. Heparinised blood was centrifuged and plasma was removed, and the erythrocytes were washed 2-3 times with saline (0.9 % NaCl) and then haemolysed in 10 volumes of cold deionized water. The whole mixture was centrifuged for 10 min at 3,000rpm. The cell debris was removed and the clear haemolysate was diluted 500 times with phosphate buffer (60mM) pH 7.4. Catalase decomposes H₂O₂ to form water and molecular oxygen. In the UV range, H₂O₂ show a continual increase in the absorption with decreasing wavelength. At 240nm, H₂O₂ absorbs maximum light. When H₂O₂ is decomposed by catalase, then the absorbance decreases. The decreased absorbance was measured at 240nm for every 15 seconds interval up to 1 min and the difference in absorbance (ΔA at 240nm) per unit time is a measure of the catalase activity. The unit of catalase activity was expressed as mM of H₂O₂ decomposed/mg Hb min⁻¹.

Plasma ceruloplasmin was measured by the Herbert and Ravin [1961] method. Ceruloplasmin oxidizes P-phenylene diamine in the presence of oxygen to form a purple-coloured oxidised product. The ceruloplasmin concentration was determined from the rate of oxidation of P-
Table 1.0
Depicts mean values and correlation coefficient \([r]\) of serum acetyl cholinesterase (AChE), C reactive proteins (CRP), liver functions tests of sprayers of vineyards before and after vitamins C (500 mg/Tab/day for 15 days) supplementation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vitamin C administered</th>
<th>Correlation Coefficient ([r])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (N=30)</td>
<td>After (N=30)</td>
</tr>
<tr>
<td>AChE [U/L]</td>
<td>4667 ± 1313 (876 – 6844)</td>
<td>4768 ± 1281** (950 – 6926)</td>
</tr>
<tr>
<td>CRP [mg/dl]</td>
<td>0.116 ± 0.082 (0.045 – 0.36)</td>
<td>0.101±0.067** (0.032 – 0.28)</td>
</tr>
<tr>
<td>AST [U/L]</td>
<td>29.40 ± 14.53 (14 – 78 )</td>
<td>26.20 ± 9.04* (17 – 54)</td>
</tr>
<tr>
<td>ALT [U/L]</td>
<td>35 ± 17.16 (14 – 88)</td>
<td>27.45 ± 10.1** (18 – 60)</td>
</tr>
<tr>
<td>TP [gm/dl]</td>
<td>7.29 ± 0.35 (6.45 – 7.9)</td>
<td>7.38 ± 0.27* (6.9 – 7.8)</td>
</tr>
<tr>
<td>ALB [gm/dl]</td>
<td>4.17 ± 0.18 (3.9 – 4.5)</td>
<td>4.29 ± 0.21** (3.9 – 4.7)</td>
</tr>
<tr>
<td>GLB [gm/dl]</td>
<td>3.17 ± 0.18 (2.8 – 3.6)</td>
<td>3.08 ± 0.17* (2.8 – 3.5)</td>
</tr>
<tr>
<td>A/G Ratio</td>
<td>1.32 ± 0.08 (1.11 – 1.46)</td>
<td>1.39 ± 0.10** (1.2 – 1.6)</td>
</tr>
</tbody>
</table>

Figures indicate Mean ± SD values and those in parenthesis are range of values.** P < 0.01, * P < 0.05, • Non significant with respect to before vitamins supplementation of the sprayers of vineyards. Acetyl cholinesterase (AChE), C Reactive proteins (CRP), Aspartate transaminase (AST), Alanine transaminase (ALT), Total proteins (TP), Albumin (ALB), Globulin (GLB).

Table 2.0
Mean values and correlation coefficient of lipid peroxide, antioxidant enzymes and trace elements of sprayers of vineyards before and after vitamin C (500 mg/Tab/day for 15 days) supplementation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Vitamin C administered</th>
<th>Correlation Coefficient ([r])</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before (N=30)</td>
<td>After (N=30)</td>
</tr>
<tr>
<td>LP [nmol/ml]</td>
<td>3.28 ±0.59 (2.27 – 5.34)</td>
<td>2.64 ± 0.41** (1.85 – 3.49)</td>
</tr>
<tr>
<td>SOD a</td>
<td>9.64 ± 1.14 (7.89 – 12.09)</td>
<td>12.03 ± 1.5*** (9.50 – 16.27)</td>
</tr>
<tr>
<td>CAT b</td>
<td>8.39 ± 4.12 (4.23 – 16.9)</td>
<td>10.12 ± 3.8** (4.22 – 18.46)</td>
</tr>
<tr>
<td>CP [mg/dl]</td>
<td>73.81 ± 12.31 (38.8 – 93.5)</td>
<td>79.25± 13.3*** (40.45 – 99.60)</td>
</tr>
<tr>
<td>GST C</td>
<td>0.099 ± 0.055 (0.022 – 0.211)</td>
<td>0.077±0.047* (0.011 – 0.22)</td>
</tr>
<tr>
<td>Serum Zn [µg/dl]</td>
<td>83.65±12.8 (61.5 – 115)</td>
<td>88.27 ± 12.5* (68 – 118)</td>
</tr>
<tr>
<td>Serum Cu [µg/dl]</td>
<td>79.90±15.3 (51 – 110)</td>
<td>84.67±15.6** (55 – 119)</td>
</tr>
</tbody>
</table>

a Unit/ml of hemolysate, b mM H₂O₂ decom/mg Hb/min, µmol of conjugate form/min/mg of protein. Figures indicate Mean ± SD values and those in parenthesis are range of values.*** P<0.001, ** P<0.01, * P<0.05, • Non significant with respect to the before vitamins supplementation of the sprayers of vineyards. Lipid peroxide (LP), RBC- Superoxide dismutase (SOD), RBC – Catalase (CAT), Plasma Ceruloplasmin (CP), Glutathione S-transferase (GST).
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phenylendiamine at 37°C at pH 6.0, which has an absorption peak at 530nm.

Serum Glutathione S-Transferase (GST) was measured by using the Habig et al., (1974) method. Glutathione S-transferase (GST) activity was determined by measuring the conjugation of 1-chloro-2, 4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation was accompanied by an increase in absorbance at 340nm. The rate of increase is directly proportional to the GST activity in the sample.

Serum zinc and copper were measured using a Perkins Elmer model 303 graphite furnace atomic absorption spectrophotometer, which was connected to a Hitachi 165 recorder; values were shown in µg dL⁻¹ [Mert and Henkin, 1971; Parson and Slavin, 1993].

Statistical comparisons between before and after vitamin C dosing of vineyard crop sprayers were made by paired t-test. Pearson’s correlation equation was also carried out to evaluate correlation between biochemical parameters of before and after vitamin C dosing.

**Results**

The mean values, percentage change, statistical significance and correlation coefficient (r) value of serum acetyl cholinesterase (AChE), C reactive proteins (CRP), liver functions tests, lipid peroxide, antioxidant enzymes and trace elements of vineyard crop sprayers and after vitamin C supplements are given in Tables 1.0 and 2.0 and Figures 1.0 and 2.0.

After 15 days of giving vitamin C supplements to vineyard crop sprayers, we observed significantly decreased serum C-reactive protein (12.78%, P<0.01, r = 0.89), aspartate transaminase (11.69%, P<0.05, r = 0.95), alanine transaminase (21.57%, P<0.01, r = 0.65), globulin (2.83%, P<0.05, r = 0.41) and increased serum acetyl cholinesterase (2.16%, P<0.01, r = 0.80), albumin (2.87%, P<0.01, r = 0.55), albumin/globulin ratio (5.3%, P<0.01, r = 0.35), whereas no statistical significant change was found in total proteins compared with before vitamin C dosing (Table 1.0 and Figure 1.0).

Vitamin C supplements given to the vineyard crop sprayers significantly decreased serum lipid peroxide (19.51%, P<0.001, r = 0.57), serum glutathione-S-transferase (21.58%, NS, r = 0.23), and increased RBC-superoxide dismutase (24.79%, P<0.001, r = 0.59), RBC-catalase (20.61%, P<0.001, r = 0.71), plasma ceruloplasmin (7.37%, P<0.001, r = 0.89), serum zinc (5.52%, P<0.01, r = 0.85), serum copper (5.96%, P<0.001, r = 0.79) compared with before vitamin C dosage (Table 2.0 and Figure 2.0).

In all above parenthesis, the percentage change, P values, and correlation coefficient (r) with respect to before vitamin C dosing of vineyard crop sprayers are given. Correlation coefficient (r) values above 0.5 of various biochemical parameters show significant correlation between before and after vitamin C supplements were given to the vineyard crop sprayers and it indicates consistency of biochemical parameters before and after vitamin C supplementation.

**Discussion**

Ascorbic acid is an important antioxidant that significantly decreases the adverse effect of reactive oxygen and oxides of nitrogen that can cause oxidative damage to macromolecules such as lipids, DNA and protein, which are implicated in chronic diseases, stroke, cancers, neuro-degenerative diseases and cataractogenesis [Halliwell and Gutteridge, 1993]. Additional dosage of L-ascorbic acid was found to be beneficial in heavy metals (nickel) induced alteration of testicular nuclear acid concentration, hepatic lipid peroxidation and histopathology of the liver [Das and Das, 2004; Das et al., 2007].

Several studies are under way to determine the effects of antioxidant supplementation following heavy metals and pesticide exposure. The data suggests that antioxidants specifically play an important role in abating certain pesticides. It is well documented that various pesticides cause membrane disruption via lipid peroxidation and alter the antioxidant status of the body, ultimately resulting cell death [Zavodnik et al., 2002; Koryagin et al., 2002; John et al., 2001] and we also found the same results in earlier studies [Patil et al., 2003, 2009b].

At the same time, supplementation of L-ascorbic acid was found to be effective in the prevention of oxidative damage in erythrocytes induced by various pesticide exposures, which resulted in significantly lowering serum lipid peroxide concentration (19.51%, P<0.001, r = 0.57) and increased RBC-SOD (24.79%, P<0.001, r = 0.59), RBC-catalase (20.61%, P<0.001, r = 0.71), and plasma ceruloplasmin (7.37%, P<0.001, r = 0.89) in vineyard crop sprayers compared with before vitamin C supplementation.
Jyotsna Patil, Dr Arun Patil, Ajit Sontakke and Sanjay Govindwar

Serum glutathione-S-transferase level was markedly increased (80.55%) before vitamin C dosage of vineyard crop sprayers as compared with the control group reported in our earlier study [Patil et al., 2009a]. However, GST activity decreased (21.58%) after vitamin C dosage of vineyard crop sprayers as compared with before vitamin C dosage.

Glutathione-S-transferase (GST) consists of a large family of GSH-utilising enzymes that play an important role in detoxification of xenobiotics in mammalian systems. Increased GST activity before vitamin C dosage in this study might be caused by the pesticides, which generally
biochemical effects of pesticides on crop sprayers in Western Maharashtra (India) vineyards before and after fifteen days of taking vitamin C supplements

combine with GSH and form less toxic, water-soluble products that are excreted in urine. Gene expression of GSH-related enzymes in human cells is increased under conditions of oxidative stress [Wang et al., 1997]. Increased regulation of GSH-dependent enzymes is necessary for an adaptive/protective response [Kaplowitz et al., 1985].

In this study, we found increased oxidative stress because of various pesticides used in vineyards. Therefore, gene expression of GST enzyme might have increased through oxidative stress, resulting in increased GST activity in this study. Vitamin C reduces oxidative stress owing to its antioxidant property. Hence, GST activity might have been decreased after vitamin C dosage because of decreased oxidative stress, which may lead to decreased gene expression of GST and reduce the GST activity.

Serum C-reactive protein level is increased significantly by 117.6% in vineyard crop sprayers as compared with the control group reported in our earlier study [Patil, 2009b]. However, CRP activity is significantly decreased (21.58%, NS, r = 0.23) after vitamin C dosage of vineyard crop sprayers compared with before vitamin C dosage.

C-reactive proteins (CRP) are serum proteins, synthesised in the liver. CRP level increases within hours of an acute injury or the onset of inflammation and may reach as high as 20 times the normal levels. Increased CRP in vineyard crop sprayers before vitamin C dosing clearly indicates the hepatic cell damage caused by various pesticides. Vitamin C may be partially preventing certain types of hepatic cellular damage [McDowell, 1989; Parola et al., 1992; Sies et al., 1992; Burtis and Ashwood, 1994; Netke et al., 1997]. In addition, vitamin C also plays a crucial role in wound healing and reducing inflammation. Therefore, CRP activity might have been decreased after vitamin C supplementation in this study.

Liver function marker enzymes, i.e. serum aspartate transaminase (AST) and alanine transaminase (ALT), were increased by 57 and 37.36% in vineyard crop sprayers respectively as compared with control subjects reported in our earlier study [Patil et al., 2009b]. However, aspartate transaminase (11.69%, P<0.05, r = 0.95) and alanine transaminase (21.57%, P<0.01, r = 0.65) were decreased after vitamin C dosage of vineyard crop sprayers compared with before vitamin C dosage. High levels of these enzymes are usually indicative of hepatic damage in this study by various pesticides.

Several procedures have been used to protect the liver from damage by administering antioxidants such as β-carotene [Olmaza and Karakilcik, 1994], vitamin C [Netke et al., 1997; Mitra et al., 1991], vitamin E [Parola et al., 1992; Harvey et al., 1994; Durak et al., 1996; Naziroglu, 1999] and selenium–vitamin E combination [Sies et al., 1992; Naziroglu, 1999; Brucato et al., 1986]. Therefore, from past reports and present results it clearly indicates that the vitamin C supplementation protects liver damage in vineyard crop sprayers, which results in decreased AST and ALT activities in this study.

In addition to its antioxidant activity, vitamin C is known to have other effects that enhance its protective effect in OP-induced toxicity. For example, vitamin C has shown to increase the activity of paraoxonase [Jarvik et al., 2002], an enzyme known to aid in the detoxification of OP and carbamate compounds.

Conclusion

The slight increase in serum AChE, albumin, and A/G ratio after vitamin C supplementation in this study might be owing to its role in protein biosynthesis, or its protective role in hepatic cell damage. Significantly decreased serum LP, GST, and increased RBC-SOD, catalase, and plasma ceruloplasmin in vineyard crop sprayers as compared with before vitamin C dosage may be owing to the antioxidant property of vitamin C. The decreased serum CRP activity in this study may be caused by vitamin C preventing hepatic cell damage, which is also supported by decreased serum AST, ALT. Therefore, the study concludes that daily intake of citrus fruits (vitamin C) is beneficial to reduce the toxicity of various pesticides on vineyard crop sprayers because of its antioxidant property, role in wound healing, reducing inflammation, preventing hepatic cellular damage and its role in protein biosynthesis.

Proper precautions like wearing protective clothes, taking a bath after pesticides spraying, wearing eye protectors and not wiping eyes with contaminated gloves or hands during spraying the pesticides will reduce the exposure to pesticides, and daily intake of antioxidant vitamins like ascorbic acid, α-tocopherol, and β-carotenes may reduce the toxicity of pesticides on vineyard crop sprayers. Minimum daily intake of two glasses of lemon juice will be very useful in decreasing adverse effects of pesticides.

In the future, a detailed study will be conducted to see the effect of these antioxidants on pesticides-exposed populations.
Acknowledgements

We express our deep gratitude to all vineyard crop sprayers who volunteered for this project. We are also thankful to vineyard owner for extending their cooperation.

References


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