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Alteration of biochemical parameters after supplementation of multivitamins and minerals of sprayers on grape gardens of Western Maharashtra (India)

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Abstract: *Background:* In the horticulture sector, the land used for cash crop like grapes is increasing particularly in Maharashtra state. There is an increasing use of the pesticides in an attempt to increase the yield and reduce the post harvest losses. The environmental pollution and poisoning due to wide use of pesticide during grape cultivation may be much more important factors to disturb the socio economical status of uneducated farm worker in rural areas. *Aim and Objectives:* The study aimed to evaluate the pesticide exposure of the population associated with grape cultivation with respect to biochemical effects, oxidative stress, antioxidants status and protecting them from such effects by supplementing vitamins A to Z. *Material and Methods:* For this study 30 subjects with occupational pesticides exposure i.e. sprayers of grape gardens having age in the range of 20 to 45 years from Tasgaon taluka, Sangli district, (Western Maharashtra) India were taken and all these study group subject,500 mg vitamin A to Z tablet / day for fifteen days were supplemented. The blood was collected by venipuncture from all these subjects for biochemical parameters assay before and after fifteen days of vitamins A to Z supplementation and all biochemical parameters were estimated by using standard methods. *Results:* Fifteen days vitamin A to Z and multi minerals supplementation to sprayers of grape gardens, we observed significant increase in serum Acetyl Cholinesterase ($P<0.001$, 2.32%), and decreased Aspartate Transaminase ($P<0.05$, 8.36%), Alanine Transaminase ($P<0.05$, 14.16%), serum Lipid Peroxide ($P<0.001$, 24.39%), Glutathione S-Transferase ($P<0.01$, 29.84%) and increased RBC-Superoxide Dismutase ($P<0.05$, 11.55%), RBC-Catalase ($P<0.001$, 25.7%), serum zinc ($P<0.01$, 3.74%), copper ($P<0.001$, 4.13%), blood lead ($P<0.01$, 3.33%) as compared to before vitamin A to Z and multi minerals supplementation and other biochemical parameters such as serum C-Reactive Proteins, Total Proteins, Albumin, Globulin, A/G ratio and Ceruloplasmin were not altered significantly as compared to before vitamin A to Z and multi minerals supplementation. *Conclusions:* Alteration of these biochemical parameters may be due to ameliorating effects of vitamin A to Z and multi minerals. The vitamins or minerals present in A to Z tablets may improve the hepatocellular damage caused by various pesticides, also beneficial for either decrease free radical formation or increased antioxidant status and increased AChE level may be decreased inhibition of AChE by pesticides of sprayers of grape gardens.

Key words: Acetyl Cholinesterase, ALT, AST, GST, CRP, Lipid Peroxide, SOD, Catalase

Introduction

The grape cultivation is increasing mainly in Maharashtra state of India and for getting more yield and reduce the post harvest loss the grape growers are spraying more pesticides. The environmental pollution and poisoning due to wide spread use of pesticide during grape cultivation may be much more important factors to disturb the socio economical status of uneducated farm worker in rural areas [1]. Pesticides are ubiquitous contaminants of our environment and have been found in air, soil, water, and human and animal tissues in samples from all over the world. Insecticides comprise a higher proportion of the total pesticide usage in developing countries than in developed countries. 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, the water-soluble pesticides are absorbed through intact skin [2]. Occupational exposures occur in the mixing, and loading of equipment and in the spraying and application of insecticides. There are several factors affecting the levels of exposures occur while mixing and handling during the agricultural application of pesticides [3], also other factors i.e. wind, equipment used, duration of exposure and individual protection affect pesticide exposure [4-5]. Absorption resulting from dermal exposure is the most important route of uptake for exposed workers. Signs and symptoms associated with mild exposures to organophosphate and carbamate insecticides include: headache, fatigue, dizziness, loss of appetite with nausea, stomach cramps and diarrhea; blurred vision associated with excessive tearing; contracted pupils of the eye; excessive sweating and salivation; slowed heartbeat, often fewer than 50 per minute; rippling of surface muscles just under the skin.

The severity of any adverse effects from exposure to pesticides depends on the dose, the route of exposure, how easily the pesticide is absorbed, the types of effect of the pesticides and its metabolites, and its accumulation and persistence in the body. Pesticides have been known to affect number of enzymes and physiological systems, which results in a wide variety of changes in human. Pesticides have been shown to affect mammalian reproduction, nervous, immune, blood coagulation system and they have carcinogenic and mutagenic potential. It affects several organs of human, but liver is most susceptible [6-7].

Depression of the respiratory centre can occur. Accumulation of acetylcholine in the CNS has believed to be responsible for the tension, anxiety, restlessness, insomnia, headache, emotional instability and neurosis, excessive dreaming and nightmares, apathy, and confusion that have been described after organophosphorus pesticide poisoning. Slurred speech, tremor, generalized weakness, ataxia, convulsions, and coma are the other CNS effects [8]. The increase formation of reactive oxygen and nitrogen species resulting increase in lipid peroxidation in several tissue mainly brain, skeletal muscle, RBC, etc. and depletes antioxidant status were reported in several studies of various pesticide exposed population.[1-6]. It is known that pesticides may irritate lung macrophages, encouraging them to generate the superoxide radical and deplete antioxidants status.

The biochemical effect produced by certain pesticides can be enzyme induction or enzyme inhibition. Several pesticides inhibits cholinesterase, altered liver and kidney functions, decreased hemoglobin, impaired oxidative stress and antioxidants imbalance and altered drug metabolism enzymes of liver have been reported among pesticide exposed workers [6-7, 9]. Therefore, the main aim of this study to evaluate the pesticide exposure of the population associated with grape cultivation with respect to biochemical effects, oxidative stress, antioxidants status and protecting them from such effects by supplementing vitamins A to Z and multi minerals.

Material and Methods

This study comprises 30 subjects with occupational pesticides exposure i.e. sprayers of grape gardens. All the study group subjects had age in the range of 20 to 45 years from Tasgaon taluka, Sangli district, (Western Maharashtra) India. For all study group subject 500 mg vitamin A to Z tablet / day for fifteen days were supplemented. The 500 mg vitamin A to Z consist vitamin C (100 mg), niacinamide (50 mg), E (25 mg), thiamine (10 mg), riboflavin (10 mg), pyridoxine (3mg), A (5000 IU), folic acid (1 mg), methylcobalamine (500 µg), pentothenate (12.5 mg) and multiminerals i. e. Zn (15 mg), Cu (2.5 mg), Mn (1.4 mg), Cr (65µg), and Se (60µg)].

Sprayers of grape gardens and grape growers were informed 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 interview. Most of the sprayers of grape gardens had major complaints of lacrimation, nausea, salivation, sniffing, headache, breathlessness, itching, vomiting. All the subjects of the study groups belong 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 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 [10]. Blood was collected by venipuncture into evacuated tubes containing heparin solution as anticoagulant from sprayers of grape gardens for biochemical parameters assay before and after fifteen days of vitamins A to Z supplementation from all subjects of 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) serum copper (Cu) and blood lead (PbB) level were measured before and after vitamins A to Z supplementation to sprayers of grape gardens by using standard methods.

Serum Acetyl Cholinesterase was measured by using Accucare kit method. The Butyrylthiocoline is hydrolysed by serum cholinesterase to produce thiocoline in the presence of potassium hexacyanoferrate (III).The absorbance decrease is proportional to the cholinesterase activity of the sample [11]. The 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 [12-13].

The liver functions tests were measured, by using a fully automated biochemistry analyzer (Eurolyser) on the same day of sample collection. The SGOT (AST) and SGPT (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 (SGOT, SGPT) reactions was measured at 340 nm, as the rate of decrease in absorbance [14]. Serum total proteins were measured by the Biuret method 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 and directly proportional to the protein concentration in the specimen [15]. Serum albumin was measured by the BCG method using reagents from M/S Beacon Ltd. Serum albumin binds with 3,3′,5,5′-tetra bromocresol sulfonapthalein (BCG) in acidic medium at pH 4.2, and the blue-green colored complex formed is measured at 600 nm [16]. Serum globulins and the A/G ratio were calculated by using serum total proteins and albumin values.

Lipid peroxidation was measured spectrophotometrically by method of Kei Satoh 1978. Serum proteins were precipitated by trichloroacetic acid (TCA) and the mixture is heated for 30 min with thioburbituric acid in 2M 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 a 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 [17]. The activity of erythrocyte superoxide dismutase (SOD) was measured by the method of Marklund and Marklund 1988. 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 420 mm. The SOD activity was measured as units mL^{-1} hemolysate. One unit of SOD is defined as the amount of enzyme required to cause 50% inhibition of pyrogallol auto-oxidation [18].

Erythrocyte catalase was measured by the method of Aebi 1983. Heparinized blood was centrifuged and plasma was removed, and the erythrocytes were washed 2-3 times with 0.9% NaCl and then lysed in 10 volumes of cold deionized water. The whole mixture was centrifuged for 10 min at 3000 rpm. The cell debris was removed and the clear hemolysate was diluted 500 times with phosphate buffer (60 mM) pH 7.4. Catalase decomposes H_2O_2 to form water and molecular oxygen. In the UV range, H_2O_2 show a continual increase in the absorption with decreasing wavelength. At 240 nm, H_2O_2 absorbs maximum light. When H_2O_2 is decomposed by catalase, then the absorbance decreases. The decreased absorbance was measured at 240 nm for every 15 seconds interval up to 1 min and the difference in absorbance (ΔA at 240 nm) per unit time is a measure of the catalase activity. The unit of catalase activity was expressed as mM of H_2O_2 decomposed / mg Hb min^{-1} [19]. Plasma ceruloplasmin was measured by method of Herbert, A., Ravin, J. 1961. Ceruloplasmin oxidizes P-phenyl-enediamine in presence of oxygen to form a purple-colored oxidized product. The cerulplasmin concentration was determined from the rate of oxidation of P-phenylenediamine at 37°C at pH 6.0, which has an absorption peak at 530 nm [20].

Serum Glutathione S-Transferase (GST) was measured by using 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 accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample [21]. Serum zinc, copper and blood lead level were measured using a Perkin Elmer model 303 graphite furnace atomic absorption spectrophotometer, which was connected to Hitachi 165 recorder; values were shown in μg dL^{-1} [22-23]. Statistical comparisons between before and after vitamins A to Z and multi minerals supplementation of sprayers of grape gardens were made by student t-test and percentage change.

Results

Table-1: Depicts mean values and percentage change of serum Acetyl Cholinesterase (AChE), C Reactive Proteins (CRP), liver functions tests of sprayers of grape gardens before and after vitamins A to Z Tab/day for 15 days supplementation.

Parameters	Vitamin A to Z		Percentage Change
	Before (N=30)	**After** (N=30)	
AChE [U/L]	4816 ± 1805 (876 – 7093)	4928 ± 1750*** (945 - 7125)	2.32
CRP [mg/dl]	0.206 ± 0.2628 (0.043 – 0.89)	0.196 ± 0.2388• (0.023 – 0.78)	-5.07
AST [U/L]	25.21 ± 7.31 (18 – 44)	23.10 ± 5.96* (19 – 44)	-8.36
ALT [U/L]	27.89 ± 7.50 (14 – 49)	23.94 ± 3.62* (16 – 31)	-14.16
TP [gm/dl]	7.18 ± 0.33 (6.7 – 7.8)	7.28 ± 0.23• (6.8 – 7.6)	1.39
ALB [gm/dl]	4.053 ± 0.189 (3.8 – 4.5)	4.062 ± 0.121• (3.8 – 4.3)	0.76
GLB [gm/dl]	3.13 ± 0.22 (2.7 - 3.6)	3.15 ± 0.32• (2.1 – 3.6)	0.63
A/G Ratio	1.29 ± 0.094 (1.08 – 1.50)	1.30 ± 0.17• (1.11 – 1.89)	0.77

Figures indicate Mean ± SD values and those in parenthesis are range of values. Acetyl cholinesterase (AChE), C Reactive proteins (CRP), Aspartate transaminase (AST), Alanine transaminase (ALT), Total proteins (TP), Albumin (ALB), Globulin (GLB),
*** P < 0.001, ** P < 0.01, * P < 0.05, • Non significant with respect to the before vitamins supplementation of the sprayers of grape gardens.

Table-2: Depicts mean values and percentage change of lipid peroxide, antioxidant enzymes and trace elements of sprayers of grape gardens before and after 15 days vitamins A to Z and multi minerals supplementation.

Parameters	Vitamin A to Z		Percentage Change
	Before (N=30)	After (N=30)	
LP [nmol/ml]	3.32 ±0.293 (2.97 – 3.69)	2.51 ± 0.33*** (1.91 – 2.83)	-24.39
Antioxidant Enzymes			
SOD [a]	9.52 ± 1.76 (5.79 – 12.62)	10.62 ± 1.96* (6.78 – 13.80)	11.55
CAT [b]	7.43 ± 2.83 (4.23 – 12.68)	9.34 ± 2.69*** (6.18 – 16.90)	25.7
CP [mg/dl]	64.85 ± 18.26 (27.91 – 85.9)	67.37± 20.36• (30.78–97.30)	3.88
GST [c]	0.110 ± 0.069 (0.020- 0.271)	0.077± 0.048** (0.019 – 0.198)	-29.84
Trace Elements			
Zn [µg/dl]	78.81 ± 16.06 (54 – 110)	81.76 ± 14.06** (55 – 108)	3.74
Cu [µg/dl]	85.21 ± 17.52 (52 – 130)	88.73 ± 16.53*** (60 =130)	4.13
Pb-B [µg/dl]	17.67 ± 6.18 (3.30 – 30)	18.26 ± 7.29** (4.5 – 35)	3.33

[a] Unit/ml of hemolysate, [b] mM H_2O_2 decom/mg Hb/min, [c] µmol of conjugate formed/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 grape gardens. Lipid peroxide (LP), RBC- Superoxide dismutase (SOD), RBC–Catalase (CAT), Plasma Ceruloplasmin (CP), Glutathione S-transferase (GST), Blood Lead (Pb- B)

Discussion

The fifteen days vitamin A to Z and multi minerals supplementation to sprayers of grape gardens, we observed significantly increased serum AChE (2.32%), and decreased AST (8.36%), ALT (14.16%) and other biochemical parameters were not altered significantly as compared to before vitamin A to Z and multi minerals supplementation might be due to ameliorating effects of vitamin A to Z tablets. The vitamin A to Z tablets consist vitamin C, E, A, niacinamide, thiamine, riboflavin, pyridoxine, folic acid, methylcobalamine, pentothenate and multi minerals i. e. Zn, Cu, Mn, Cr, Se. Therefore, exactly it is not clear which vitamins or minerals plays crucial role for decreased AST, ALT and slight increased AChE levels. We found vitamin C and E supplementation decreased these transaminase levels [24-25].

Therefore, A to Z vitamins or minerals may improve the hepatocellular damage caused by various pesticides, and increased AChE level may be decreased inhibition of AChE by pesticides. Significant decrease in serum lipid

peroxide (24.39%), GST (29.84%) and increased levels of RBC-SOD (11.55%), RBC-catalase (25.7%), serum zinc (3.74%), copper (4.13%), blood lead (3.33%) were observed after fifteen days supplementation of multivitamins and minerals as compared to before vitamin A to Z supplementation. Serum lipid peroxide level was decreased in this study might be due to vitamin C, E and zinc, copper elements. The zinc, copper activates SOD and selenium GSHPx. Increased RBC-SOD, RBC-catalase might be due to decreased generation of free radicals or oxidative stress by antioxidant vitamins and minerals supplementation in this study.

Catalase level was increased more than SOD may be due to increase GSHPx activity or increase heme biosynthesis. Vitamin C, B_6 and Cu stimulates heme biosynthesis, which are present in vitamin A to Z. Therefore, vitamin A to Z and multi minerals supplementation is beneficial for either decrease free radical formation or increased antioxidant status of sprayers of grape gardens.

Zinc has been shown to have an antioxidant potential through the non-enzymatic stabilization of biomembranes and biostructures [26] have shown that CCl_4 induced lipid peroxidation in the microsomal fraction of liver homogenates was inhibited by adding zinc to the incubation medium. The slight increased serum zinc and copper levels in this study might be due supplementation of multi minerals i.e zinc, copper, Mn, Cr, Se, which are present in A to Z vitamin tablets or may be vitamin A to Z increased absorption of these elements. Increased slight blood lead may due to increased absorption or release accumulated lead from soft tissue. Therefore, supplementation of multivitamins and minerals are beneficial to reduce the hepatotoxicity and also useful to maintain the oxidative/antioxidant balance in sprayer of grape gardens caused due to various pesticides.

Sprayers of grape gardens should take proper precaution while spraying the pesticides on grape gardens. They should wear protective clothing and spraying should be done during periods when there is little or no wind. They should change contaminated clothes immediately and wash affected areas of the skin. They should take bath after pesticides spraying and wear clean clothes at the start of each day during pesticide application. They should always wear eye protection when pesticide sprays and should not wipe eyes with contaminated gloves or hands. Pesticides should not be sprayed on grape gardens when air temperatures are above 30°C. Prevention is always better to reduce pesticide exposure. Taking natural antioxidants such as lemon juice, fruits, carotenes, leafy vegetables and fish is beneficial to reduce pesticide toxicity. Minimum daily intake of two glass of lemon juice will be very useful to decrease adverse effects of pesticides. During spraying seasons every fifteen days medical health check up of sprayers of grape gardens is essential.

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