Protection of Liver Injury by Vitamin C, E and GSH after Methomyl Toxicity in Rat

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ABSTRACT

Methomyl (Lannate), a carbamate pesticide induces liver injury by increasing lipid peroxide, superoxide dismutase and inhibiting microsomal cytochrome P₄₅₀ which is prevented by supplementation of vitamins. Aims and objectives: To study effect of supplementation of vitamins C, E and Glutathione (GSH) on lipid peroxide and superoxide dismutase, mixed function oxidase in methomyl treated rats. Materials and Methods: Adult male rats (weighing 200-230 g) were divided into 4 groups each of 6 animals. Animals from group 2, 3 and 4 were given a dose of 1, 2 and 4 mg methomyl/kg body weight (bw) intra peritoneal, respectively for 3 consecutive days. Second set of experiment adult male rats were divided into 4 groups. Animals from group 2, 3, 4 were injected selected dose methomyl (4mg/kg bw) for 1, 3 and 5 successive days. Third set of experiment adult male rats were divided into 5 groups. Animals from group 2 were injected methomyl 4mg/kg bw. Animals from group 3, 4, and 5 were injected methomyl (4mg/kg bw) along with Vitamin C, E and GSH (100 mg/kg bw each) respectively. Group-1 of each category received an equivalent amount of saline as control. Results: Increased dose and duration of methomyl treatment to rats increases the microsomal LP and SOD. Supplementation of vitamin C, E and GSH (100mg/kg bw each) to methomyl pretreated groups received in saline water separately and observed that microsomal LP and SOD was significantly increased in methomyl (P<0.01 and P<0.05) treated rats and decreases in supplementation of vitamin C, E and GSH to methomyl-pretreated rats. Group-1 injected 0.9% saline and served as control in all the experiment. alteration in microsomal mixed function oxidases observed in Methomyl toxicity. Protection of liver is observed after supplementation of vitamin C, E and GSH on mixed function oxidases.

Keyword: Methomyl; vitamin C; Vitamin E; Glutathione (GSH); Oxidative Stress; Lipid Peroxidation; Mixed Function Oxidase
INTRODUCTION

Lannate is widely used for the control of a large variety of insects (leafhoppers and thrips), on a wide range of crops viz. fruits, vines, hops, vegetables, grains soybeans, cotton and ornamentals throughout the world [1]. The active ingredient in lannate is a methomyl, (S-methyl-N-(methylcabomyl)-thioacetimidate), a compound of the oxime carbamate group. Methomyl has been classified as a pesticide of category-I toxicity [2]. It is an insecticide of low chronic, but high acute toxicity. This acts by direct contact or following ingestion through the stomach.

Metabolic pathway for methomyl in the rat includes the displacement of the S-methyl moiety by glutathione and enzymatic transformation to produce a mercapturic acid derivative. Another pathway involves hydrolysis to give S-methyl-N-hydroxy thioacetimidate, which is rapidly broken down to carbon dioxide [1]. Methomyl treated rats showed histopathologic changes in kidney and spleen of male and female rats. Similarly, enzymatic alterations of acetyl cholinesterase and liver glucose-6-phosphate dehydrogenase were also observed [3].

Microsomal cytochrome P450 (CYP450) consists of multigene family that plays important role in the metabolism of a wide variety of endogenous compounds and xenobiotics including drugs, carcinogens, toxic chemicals, steroids and fatty acids [4]. Methomyl is known as a potent cholinesterase inhibitor. A decrease in the level of cytochrome P450 and the activity of drug metabolizing enzymes at higher dose level in male rats indicates destruction of cytochrome P450. Decreased hemoglobin content indicates that methomyl may involve in heme synthesis. A significant increase in liver enzymes (ALT, AST and ALP) indicates liver damage [5]. It is established that many pesticides, in common use, can produce some toxic and adverse effects on the liver, kidney and other biological systems, when tested on various types of experimental animals through their mode of action or by production of free radicals that damage all cell components [6]. Pesticide chemicals can induce oxidative stress by generating free radicals and altering antioxidant levels of the free radical scavenging enzyme activity [7]. Exposure to endosulfan and chlorpyrifos can differentially modify endogenous antioxidants like SOD, GPX and GSH, which can lead to the development of oxidative stress in some tissues [8]. Chlorpyrifos intoxication causes a significant decrease in the reduced Glutathione (GSH), Catalase (CAT) and Glutathione–S-Transferase (GST) activities [9].

A major contributor to non-enzymatic protection against lipid peroxidation is vitamin C and E, well known free radical scavengers [10]. Vitamins E, C and GSH are antioxidants which exert their effect by curbing the excess free radicals formation and thereby controlling MDA levels which indicate lipid peroxidation.

The genesis of present study is to see whether the antioxidants supplementation help to alleviate the oxidative stress in pesticide toxicity in experimental animals which may have implications in managing human who exposed to pesticide during spraying on grape gardens.

MATERIAL AND METHODS

Chemicals and Reagents

Methomyl (Lannate) was obtained from Du point, USA; 2-thiobarbituric acid (2, 6-dihydroxypyrimidine-2-thio; TBA) from Merck. Reduced Nicotinamide Adenine Dinucleotide Phosphate (NADPH), Oxidized Nicotinamide Adenine Dinucleotide Phosphate (NADP), Cytochrome c, Glucose 6-Phosphate, Glucose 6-Phosphate Dehydrogenase, Aminopyrine, Aniline Hydrochloride, Malondialdehyde (MDA), Pyrogallol, Glutathione (reduced) were obtained from Sigma Chemical Co. (St. Louis MO), Sucrose, Phenol, Trichloroacetic Acid, Sodium Chloride, Potassium Chloride, Calcium Chloride and other chemicals were of analytical
grade, obtained from Qualigens Chemical (Bombay). Vitamins E and C are from local medical stores.

Animals
Three month old male (weighing 200-230 g, 80-100 days) Wistar rats were obtained from Haffkine Institute, Bombay, India. The animals were housed in standard cages and were given an appropriate standard laboratory diet (Hindustan Lever Ltd, Bombay) and tap water ad libitum. The animals were used after clearance from the Institutional Animal Ethics committee.

Experimental Groups
Adult male rats (weighing 200-230 g) were divided into 4 groups each of 6 animals. Animals from group 2, 3 and 4 were given a dose of 1, 2 and 4 mg methomyl/kg body weight intra peritoneal, respectively for 3 consecutive days. In the second set of experiment adult male rats were divided into 4 groups. Animals from group 2, 3, 4 were injected methomyl (4mg/kg bw) for 1, 3 and 5 successive days. In the third set of experiment adult male rats were divided into 5 groups. Animals from group 2 were injected methomyl 4mg/kg body weight. Animals from group 3, 4, and 5 were injected methomyl (4mg/kg bw) along with Vitamin C, E and GSH (100mg/kg bw each) respectively. Group-1 of each category received an equivalent amount of saline as control. The volume injected into rats of body weight 200 g was 1 ml. between 8:00 am and 9:00 am.

Preparation of Microsomes
The rats used in this study were killed 24 hr after the last dose by cervical dislocation. Their livers were perfused in situ with ice cold 1.15% KCl containing 0.05 mM EDTA, rapidly excised, blotted dry, weighed, minced and homogenized with 2 volumes of ice cold 0.25 M sucrose solution, in a Potter–Elvehjem type homogenizer. The homogenate was centrifuged at 10,000 x g for 10 min in a refrigerated centrifuge (REMI C-24). The microsomes were isolated by the procedure of Cinti et al [11]. The microsomal pellet was suspended in phosphate buffer (0.1 M, pH 7.4) and the suspension was used for microsomal enzyme assays. Microsomal protein content was measured by the Biuret method using bovine serum albumin as standard [12].

Enzyme assays
The levels of microsomal electron transport components, cytochrome P_{450} and cytochrome b_{5} were determined using Hitachi UV-visible recording spectrophotometer by the procedure of Omura and Sato [13, 14]. Cytochrome c reductase activity was determined by the method of Masters et al [15]. Aminopyrine N-demethylase activity was assayed according to the procedure of Schenkman et al [16]. Formaldehyde liberated during N-demethylation was estimated by the procedure of Nash [17]. Aniline hydroxylase assay was performed using the procedure reported by Govindwar and Dalvi [18].

Biochemical Assay
The biochemical measurements in microsomes were performed according to method given below in brief.

Assay of Superoxide Dismutase Activity
The measurement of superoxide dismutase (SOD; EC1.15.1.1) is based on the principle in which superoxide anion is involved in autoxidation of pyrogallop at alkaline pH 8.5. The SOD inhibits the autoxidation of pyrogallop, which can be determined as an increase in the absorbance per 2 min at 420 nm (1988). SOD activity was expressed in units / mg protein [19].
Estimation of Hepatic Microsomal Lipid Peroxide

Kohn and Liver sedge described the colorimetric reaction of thiobarbituric acid (TBA) with an unknown substance forming during the aerobic incubation of tissue homogenates. Patton and Kurtz later identified this as malondialdehyde (MDA), a secondary product of lipid peroxidation. The reaction of lipid peroxides with TBA has been widely adopted as a sensitive assay method for lipid peroxidation in animal tissues. Yagi reported an assay method for lipid peroxides in blood plasma by TBA reaction under optimum conditions, the reaction conditions especially the pH of the reaction mixture must be carefully determined while measuring lipid peroxide level in animal tissues. Optical density of organic layer measured at 532nm on spectrophotometer against blank. The concentration of MDA calculated by using standard graph [20].

Spectrophotometric measurements

The spectrophotometric measurements were performed by using a Hitachi uv-visible recorder (Japan).

Statistics

Statistical analysis was done by using one-way analysis of variance and Tuckey krammer post test. The level of significance was set at 0.05.

RESULTS

Administration of methomyl at different dose for three days resulted in significant increase in microsomal lipid peroxidation (LP) and superoxide dismutase (SOD) level by 26.66%, and 16.71% at 1 mg/kg, 41.11%, and 23.58% at 2 mg/kg, 48.33%, and 41.12% at 4 mg/kg body weight (Table 1, fig. 1).

Selected dose of methomyl (4mg/kg bw) injected for different days resulted in significant increase in lipid peroxidation and SOD by 44.51% and 14.29% at 1 day, 63.94% and 32% at 3 days, 114 % and 34% at 5 days of adult rats as compared to control rats (Table 2, Fig. 2). Microsomal LP and SOD was significantly increased by 36.22%, and 22.89% in methomyl, and antioxidants supplementation showed that increase LP and SOD by 18.87%, and 7.39% in methomyl + vitamin E, 14.03%, and 9.96% in methomyl + vitamin C, 23.46%, and 11.44% in methomyl + GSH treated rats, respectively as compared to control rats. Microsomal proteins was significantly decreased by 37.25% at methomyl treated, 35% at methomyl + vitamin E, 16.47% at methomyl + GSH and 14.29% at methomyl + vitamin C, treated rats as compared to control rats. Microsomal cytochrome b5, and cytochrome P450, levels were significantly inhibited by 43.28%, and 33.33% due to methomyl, 27.27% and 23.23% by methomyl + vitamin E, 12.12 % and 27.27% by methomyl + GSH, 24.24% and 23.23% by methomyl + vitamin C, treated rats respectively as compared to control (Table 3, 4). These results indicate that the microsomal protein decreased more in methomyl treated rats as compared to vitamin C and GSH supplementation to methomyl-pretreated rats. Microsomal cytochrome b5 and cytochrome P450 levels were significantly inhibited by 43.28%, and -33.33% due to methomyl, 27.27% and 23.23% by methomyl + vitamin E, 12.12 % and 27.27% by methomyl + GSH, 24.24% and 23.23% by methomyl + vitamin C, treated rats respectively as compared control rats (Table 3,4). Whereas, no significant change was observed in case of cytochrome c reductase in all the three groups except methomyl treated rats increased by 25.91% as compared to control rats. Drug metabolizing enzymes i.e. aminopyrine N-demethylase, and aniline hydroxylase were significantly decreased by 43.7% and 29.31% due to methomyl, 30.14 %and 24.13% by methomyl + vitamin E, 21.37% and 18.96% by methomyl + GSH, and 28.07% and 21.12%, by methomyl + vitamin C, treated rats respectively as compared to control rats (Table 3,4).
Table 1: Effect of Different Doses of Methomyl on Microsomal Lipid Peroxide and Superoxide Dismutase of Adult Rats. [Each Group Consist Six Rats]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>1 mg/kg</th>
<th>2 mg/kg</th>
<th>4 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP *</td>
<td>3.6 ± 0.28</td>
<td>4.56 ± 0.25**</td>
<td>5.08 ± 0.68***</td>
<td>5.34 ± 0.45****</td>
</tr>
<tr>
<td>SOD b</td>
<td>29.1 ± 4.33</td>
<td>34 ± 2.75**</td>
<td>36 ± 2.36***</td>
<td>41.11 ± 1.7***</td>
</tr>
</tbody>
</table>

Values are means of three experiments ± SD * P < 0.01, ** P < 0.001, Lipid peroxide (LP), Superoxide dismutase (SOD). * nmol/mg of microsomal protein, b Unit/mg of microsomal proteins.

Fig. 1: Percentage Change of Effect of Different Doses of Methomyl on Microsomal Lipid Peroxide and Super Oxide Dismutase of Adult Rats. [Reference Table - 1]

Table 2: Effect of Different Duration of Methomyl Doses on Microsomal Lipid Peroxide and Superoxide Dismutase of Adult Rats.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control group</th>
<th>1 day</th>
<th>3 days</th>
<th>5 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>LP *</td>
<td>3.19 ± 0.42</td>
<td>4.61 ± 0.42**</td>
<td>5.23 ± 0.24***</td>
<td>6.84 ± 0.40****</td>
</tr>
<tr>
<td>SOD b</td>
<td>29.38 ± 2.01</td>
<td>33.58 ± 1.10*</td>
<td>38.81 ± 1.97**</td>
<td>39.63 ± 1.93***</td>
</tr>
</tbody>
</table>

Values are means of three experiments ± SD * P < 0.05, ** P < 0.01, *** P < 0.001, Lipid peroxide (LP), Superoxide dismutase (SOD). * nmol / mg of microsomal protein, b Unit /mg of microsomal proteins.
**Fig. 2:** Percentage Change of Effect of Duration of Methomyl Dose on Microsomal Lipid Peroxide and Superoxide Dismutase of Adult Rats. [Reference Table – 2]

1 day, 3 days 5 day Lipid peroxide (LP), Superoxide dismutase (SOD).

**Table 3:** Alteration in Male Rats Liver Microsomal Protein, Electron Transport Components and Drug Metabolizing Enzymes, Lipid Peroxide, Superoxide Dismutase Due to Methomyl (4 mg/Kg), Vitamin E (100 mg/kg) + Methomyl (4 mg/kg), Vitamin C (100 mg/kg)+ Methomyl (4 mg/Kg) and GSH (100 mg/kg) + Methomyl (4 mg/kg) of Three Days Treatments

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Methomyl</th>
<th>Vit E+Met</th>
<th>GSH+Met</th>
<th>Vit C+ Met</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP a</td>
<td>14.2±2.73</td>
<td>8.91 ± 0.62</td>
<td>9.23 ± 0.47</td>
<td>11.86 ±1.39</td>
<td>12.17 ± 1.51</td>
</tr>
<tr>
<td>(10 - 17.9)</td>
<td>(8.2 - 9.9)</td>
<td>(8.5 - 9.7)</td>
<td>(9.9 - 14.10)</td>
<td>(10.22-13.30)</td>
<td></td>
</tr>
<tr>
<td>Cyt b5 b</td>
<td>0.33 ± 0.06</td>
<td>0.19 ±0.04***</td>
<td>0.24 ±0.07**</td>
<td>0.29 ± 0.072*</td>
<td>0.25±0.023**</td>
</tr>
<tr>
<td>(0.26 – 0.40)</td>
<td>(0.14 – 0.24)</td>
<td>(0.14 – 0.34)</td>
<td>(0.21 – 0.38)</td>
<td>(0.22 – 0.28)</td>
<td></td>
</tr>
<tr>
<td>Cyt P450 c</td>
<td>0.49 ± 0.054</td>
<td>0.33± 0.056***</td>
<td>0.38 ±0.034*</td>
<td>0.36 ± 0.05**</td>
<td>0.38 ± 0.064*</td>
</tr>
<tr>
<td>(0.42 – 0.57)</td>
<td>(0.28 – 0.40)</td>
<td>(0.32 – 0.42)</td>
<td>(0.26 – 0.45)</td>
<td>(0.27 – 0.49)</td>
<td></td>
</tr>
<tr>
<td>Cyt C red d</td>
<td>22.50 ± 2.73</td>
<td>28.33 ± 2.58**</td>
<td>24.80 ± 2.67</td>
<td>23.83 ± 2.71</td>
<td>25.16 ± 3.25</td>
</tr>
<tr>
<td>AND e</td>
<td>6.27 ±1.64</td>
<td>3.53 ± 1.30**</td>
<td>4.38 ±0.577*</td>
<td>4.93 ± 1.17*</td>
<td>4.51 ± 1.10*</td>
</tr>
<tr>
<td>(4.18 – 8.40)</td>
<td>(1.90 – 5.4)</td>
<td>(3.80 – 5.40)</td>
<td>(2.90 – 6.50)</td>
<td>(2.70 – 5.60)</td>
<td></td>
</tr>
<tr>
<td>AH f</td>
<td>2.32 ± 0.589</td>
<td>1.64 ± 0.498*</td>
<td>1.76 ± 0.44*</td>
<td>1.88 ± 0.343*</td>
<td>1.83 ± 0.541*</td>
</tr>
<tr>
<td>(1.38 – 2.90)</td>
<td>(1.0 – 2.30)</td>
<td>(1.20 - 2.30)</td>
<td>(1.40 – 2.40)</td>
<td>(1.40 – 2.6)</td>
<td></td>
</tr>
<tr>
<td>LP e</td>
<td>3.92 ± 0.76</td>
<td>5.34 ± 0.37**</td>
<td>4.66 ± 0.62*</td>
<td>4.84 ± 0.69*</td>
<td>4.47 ± 0.53*</td>
</tr>
<tr>
<td>(2.96 – 4.89)</td>
<td>(4.9 – 5.90)</td>
<td>(3.96 – 5.40)</td>
<td>(3.90 – 5.80)</td>
<td>(3.74 – 5.20)</td>
<td></td>
</tr>
<tr>
<td>SOD h</td>
<td>45.16 ± 9.94</td>
<td>55.50 ± 10.5*</td>
<td>48.50 ± 5.82*</td>
<td>50.33 ± 7.96*</td>
<td>49.66 ± 8.45*</td>
</tr>
</tbody>
</table>

Values are means of three experiments ± SD; six animals in each group. a mg of protein/g liver, b nmole/mg of microsomal protein, c nmole cytochrome c reduced/min/mg of microsomal protein, d nmole formaldehyde liberated/min/mg of microsomal protein, e nmole p-aminophenol formed/min/mg of microsomal protein, f nmol/mg of microsomal protein, g Unit/mg of microsomal proteins.
Microsomal proteins (MP), Cytochrome b₅ (Cyt b₅), Cytochrome P₄₅₀ (Cyt. P₄₅₀) Cytochrome c reductase (Cyt C red), Aminopyrine N-demethylase (AND), Aniline hydroxylase (AH), Lipid peroxide (LP), Superoxide dismutase (SOD).

* P < 0.05, ** P < 0.01, *** P < 0.001, * Non signficant with compared to control.

Table 4: Percentage Change of Alteration in Rats Liver Microsomal Protein, Electron Transport Components and Drug Metabolizing Enzymes, Lipid Peroxide, Superoxide Dismutase due to Methomyl (4 Mg /Kg), Vitamin E (100 Mg/Kg) + Methomyl (4 Mg/Kg), Vitamin C (100 Mg/Kg) + Methomyl (4 Mg/Kg) And GSH (100 Mg/ Kg) + Methomyl (4 Mg/Kg) of Three Days Treatments With Respect to Control Group Rats.

<table>
<thead>
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<th>Parameters</th>
<th>Treatments</th>
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<tr>
<td></td>
<td>Methomyl</td>
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<tr>
<td>MP a</td>
<td>-37.25</td>
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<tr>
<td>Cyt b₅ b</td>
<td>-43.28</td>
</tr>
<tr>
<td>Cyt P₄₅₀ c</td>
<td>-33.33</td>
</tr>
<tr>
<td>Cyt C red d</td>
<td>25.91</td>
</tr>
<tr>
<td>LP g</td>
<td>36.22</td>
</tr>
<tr>
<td>SOD h</td>
<td>22.89</td>
</tr>
</tbody>
</table>

a mg of protein/g liver, b nmole/mg of microsomal protein. c nmole cytochrome c reduced/min/mg of microsomal protein. d nmole formaldehyde liberated/min/mg of microsomal protein. e nmole p-aminophenol formed/min/mg of microsomal protein. g nmol/mg of microsomal protein, h Unit/mg of microsomal proteins [Ref. table 3].

DISCUSSION

In the present study methomyl treatment resulted in increased oxidative stress in the rat as evidenced by enhanced levels of thiobarbituric acid reactive substancse (TBARS), accompanied by concomitant increase in the levels of superoxide scavenging enzymes SOD, in liver. The increase levels are dose and duration dependent. Increase formation of reactive oxygen and nitrogen species resulting an increase in the lipid peroxidation in several tissues mainly brain, skeletal muscle, RBC, etc. A deplete antioxidant status was reported in several studies of various pesticide exposed population [21- 25]. However, in case of short-term pesticides exposure, the antioxidants enzymes are increased due to more generation of free radicals, and in case of long-term pesticides exposure, the antioxidants enzyme levels are depleted due to continuous utilization for scavenging the reactive oxygen species. We observed increased SOD level may be short-term methomyl exposure. Pesticides may increase oxidative damage because they are more active to oxygen free radical that re-oxidizes to make superoxide or the pesticides may itself be free radicals or they may deplete antioxidants defences. Paraquat is reduced free radical that reoxidizes to make a superoxide and regenerate paraquat, which accumulates selectively. The overall effect of pesticides is the production of more free radicals [26]. Pesticides may irritate lung macrophages, encouraging them to generate
the superoxide radical. The organisms use antioxidant enzymes as natural endogenous protection against the generation of oxygen species [27].

Superoxide dismutase is an antioxidant enzyme that catalyses the dis-mutation of the highly reactive superoxide anion to O$_2$ and the less reactive species H$_2$O$_2$. Peroxide can be destroying by catalase or Glutathione Peroxide reactions [28]. In humans, there are three forms of SOD, 1) Cytosolic: Cu/Zn SOD 2) Mitochondrial: Mn-SOD, and 3) Extra cellular SOD [29]. If there is an increase in the production of free radicals in the body, extra antioxidants produced. However, if large numbers of extra free radicals produced results cell damage and death, due to imbalance between free radicals and antioxidants [26].

Selected dose of methomyl treatment at 4mg/kg bw for 3 days decreases in cytochrome P$_{450}$ content and the activity of drug metabolising enzymes at dose and duration dependent manner in male rats indicate destruction of cytochrome P$_{450}$, this coincides with our earlier report. Many pesticides including organochlorine and organophosphorous compounds have been reported to inhibit the activity and alteration in the expression of various cytochrom P$_{450}$ isoforms during its oxidative biotransformation. These changes may increase the sensitivity of cell against reactive endogenous metabolites or other xenobiotics [30].

Methomyl treatment to rats increased microsomal LP due to more generation of free radicals and to scavenge these radicals, microsomal SOD activity increased. The increase microsomal LP and SOD decreases by supplementation of vitamin E, C, and GSH may be due to antioxidants properties of these compounds. Vitamin E acts as a chain breaking antioxidants for lipids in biological membranes [31, 32]. The GSH is an endogenous thiol antioxidant that has a multifaceted role in xenobiotic metabolism and is a first line of defence against oxidant-mediated cell injury [33].

Present result indicate that the microsomal protein decreased more in methomyl treated rats as compared to vitamin C and GSH supplementation to methomyl pre-treated rats (Table 4). From this, it is clear that the vitamin C and GSH may prevent the adverse effects of methomyl on protein biosynthesis, or it may have some role in protein biosynthesis. Several studies reported that the methomyl inhibits the protein biosynthesis [34, 35]. and induces oxidative stress. In oxidative stress, decreased protein biosynthesis has well documented in literature. In this study, vitamin C and GSH may be involved in scavenging the free radicals generated by methomyl and decreases the oxidative stress and indirectly preventing the inhibition of protein biosynthesis by methomyl in rats.

Vitamin C enhances the synthesis of immunoglobulin (antibodies) and increase the phagocytic action of leucocytes and peptide hormone synthesis. Many peptide hormones contain carboxyl terminal amide, which is derived from terminal glycine. Vitamin C is required for hydroxylation of glycine carried by peptidylglycine hydroxylase. The vitamin C is also involved in collagen, ferritin, serotonin and carnitine synthesis [31, 32]. From these vitamin C functions, it clearly understood that the vitamin C might enhance the microsomal proteins biosynthesis. Glutathione supplementation to methomyl-pre-treated rats increases the microsomal proteins as compared to methomyl treatment. It could be explain by the role of glutathione in “γ-glutamyl cycle” for absorption of amino acids from gut [33, 34]. Glutathione enhance amino acid absorption across the gut, which may be indirectly accelerating the microsomal protein biosynthesis.

Ascorbic acid seems to take part in electron transport system of mammalian ‘microsomes’, due to its easy oxidation with reversible
reduction of ascorbic acid. Enzyme like ascorbic acid oxidase, cytochrome oxidase, flavine transhydrogenase participate in the electron transport system, where ascorbic acid takes part between NADH and cytochrome b5. The detail mechanism of the role of vitamin C is not known, but suggested that the coupled reaction with hydroxylation [31]. Therefore, vitamin C supplementation may be restored the cytochrome b5 activity in case of methomyl pre-treated rats. Oxidative modification of microsomal protein produced by NADPH-450 reductase/P450/O2system is exclusively prevented by ascorbic acid this may be explained by the consideration that perferryl radical, P450-Fe3+O2 is reduced and inactivated by ascorbic acid and not by GSH, alpha tocopherol (Vit E) and other antioxidants used [35].

Microsomal cytochrome P450 enzyme activity has reduced in methomyl treated rats may be due to inhibition of various cytochrome P450 isoforms or it may be due to inhibition of heme biosynthesis, resulting the decrease activity of various cytochromes. Vitamin C supplementation to methomyl pre-treated rats increased the microsomal cytochrome b5 and cytochrome P450 activity may be the enhancing the heme biosynthesis. Ascorbic acid enhances iron absorption by keeping it in the ferrous form due to its reducing property. It also helps in the formation of ferritin (storage form of iron) and mobilization of iron from ferritin, which is required for heme biosynthesis [31, 32]. Lead arsenate (di plumbic hydrogen arsenate) used as insecticide, and fungicide. The arsenic trioxide depletes ascorbic acid [36]. Deficiency of ascorbic acid inhibits cytochrome content, and microsomal mixed function oxidases reported in literature [37].

In earlier discussion, we have seen that vitamin C increasing the hepatic microsomal protein biosynthesis. Moreover, our results are consistent with others report in literature. Glutathione, a thiol tripeptide, is the most important endogenous antioxidant that is found in mM concentrations in tissues and is responsible for maintaining the cellular redox state with smaller thiols such as thioredoxin, glutaredoxin and peroxiredoxin [38].

Methomyl mainly detoxifying by conjugation with GSH forming mercapturic acid that excrete in urine. Increased microsomal protein, cytochrome b5 and cytochrome P450, aminopyrine N-demethylase, aniline hydroxylase enzymes activity after supplementation of GSH to methomyl pretreated rats may be due to immediate conjugation of methomyl by GSH, reduces its adverse effects on mixed function oxidase and drug metabolizing enzymes along with decrease oxidative stress by improving antioxidant status.

Increased cytochrome b5 and cytochrome P450, aminopyrine N-demethylase, aniline hydroxylase enzymes activity after supplementation of Vit E to methomyl pretreated rats may due to the role of vitamin E as an antioxidant. Vit E allows free radicals to abstract a hydrogen atom from the antioxidant molecule rather than from polyunsaturated fatty acids, thus breaking the chain of free radical reactions, the resulting antioxidant radicals being a relatively unreactive species [39].Thus vit E inhibiting the formation of free radical, and decreases the methomyl toxicity.

The basis of pesticide toxicity in the production of reactive oxygen species may be due to:

1) Their “redox-cycling” activity-they readily accept an electron to form free radicals and then transfer them to oxygen to generate superoxide anions and hence hydrogen peroxide through dismutation reaction.

2) Generation of free radicals probably because of the alteration in the normal hemostasis of the body resulting in oxidative stress, if the requirement of continuous antioxidants is not maintained [40].
The efforts of the endogenous antioxidant enzymes to remove the continuously generated free radicals initially increase due to an induction but later enzyme depletion results, resulting in oxidative cell damage [41]. In case of short-term pesticides exposure, the antioxidants enzymes are increased due to more generation of free radicals, and in case of long-term pesticides exposure, the antioxidants enzyme levels are depleted due to continuous utilization for scavenging the reactive oxygen species. Present study can conclude that supplementation of Vitamin C, E and glutathione decreases oxidative stress and improves antioxidant SOD in methomyl exposed rats. Antioxidant role of these may be one of the key mechanisms of its protective effect.

CONFLICT OF INTEREST STATEMENT
The authors declare that they have no competing interests.

REFERENCES


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