Modulation of ethion-induced hepatotoxicity and oxidative stress by vitamin supplementation in male Wistar rats

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ABSTRACT

Organophosphorus insecticides (OPIs) may induce oxidative stress leading to generation of free radicals and alteration in antioxidant system of animals. Many studies reported that enzymatic and non enzymatic antioxidant may play protective role against OPIs induced toxicity in human and rats, of present study was to investigate the possible protective role of vitamin E on ethion-induced toxicity in rats using qualitative, quantitative and biochemical approaches. Adult male albino rat strain were randomly divided into four groups; each group consists of six animals. Animals were treated for a period of 28 days. Group I (control group received corn oil); Group II [ethion (2.7 mg/kg bw/day)]; Group III (vitamin E treated (50 mg/kg of bw/day)]; Group IV (ethion + vitamin E treated). Animals were sacrificed after 7, 14, 21 and 28 days by decapitation and liver tissue was collected for the measurement of proteins, lipid peroxidation (LPO), reduced glutathione (GSH) content and activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) and glutathione-S-transferase (GST). Erythrocytes were analysed to measure acetyl cholinesterase activity. The result of this study shows that in vivo administration of ethion in rats caused significant induction of oxidative damage in liver tissue as evidenced by increased level of proteins and decreased GSH content. Ethion toxicity also led to a significant increase in the activities of SOD, CAT, GPx and GST in liver tissue. In addition, decrease in GR activity was observed in ethion administered compared to control. Histopathological findings revealed that exposure to ethion caused damage to liver tissue. However, simultaneous supplementation with vitamin E restored these parameters par excellence in comparison to control. The results of the current study revealed that ethion-induced toxicity caused lipid peroxidation, alterations in the antioxidant enzymes and histopathological changes in liver. Supplementation of vitamin E exhibited protective effect by inhibiting ethion-induced toxicity in liver and erythrocyte.

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1. Introduction

Several hundred pesticides have been synthesized and used in agricultural practices in order to enhance food production by eradicating unwanted insects and controlling disease vectors. Because of widespread use and easy accessibility, poisoning with organophosphates (OP) has become a global health problem in both developing and developed countries [1–3]. Recent extrapolations of data from a few countries in Asia suggest that every year there are three million cases of severe poisoning and 2,20,000 deaths [4–6]. Currently, India is the largest producer of pesticides in Asia and ranks twelfth in the world for the use of pesticides. A vast majority of Indian population is engaged in agriculture as cultivators, farm owners and laborers. People are directly exposed to pesticides through dermal contact and inhalation and indirectly through the food chain.

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Organophosphate insecticides (OPIs) are one of OP class that is widely used to kill insects. OPIs poisoning is unfortunately common and many fatal cases are reported [7–10]. OPIs are known to cause inhibition of acetyl cholinesterase (AChE) activity in target tissues which accumulates acetylcholine and paves way for smooth transmission of nerve functions [11]. In chronic cases of exposure to organophosphates, inhibition of oxidative stress has been reported as the main mechanism of toxicity in many studies [1,12–14]. Various experimental studies have shown that oxidative stress in biological systems originates as a result of imbalance between the generation of oxidizing radicals and cellular antioxidant defenses [1,12–29]. Oxidative stress may lead to excessive generation of reactive oxygen species (ROS) and alterations in antioxidant and the scavenging enzymes [30]. Subchronic exposure of rats to malathion has shown enhancement of ROS and lipid peroxidation in saliva, plasma and liver of treated animals [12,13].

The cells have many ways to alleviate the effects of oxidative stress, either by repairing the damage or by directly dimi
the occurrence of oxidative damage by means of enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants include superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase etc. The non-enzymatic scavengers include melatonin, selenium, glutathione (GSH), vitamin E and C etc. Recent studies have shown that supplementation of vitamin E plays a protective role against OPI-induced toxicity in rats and other animals [18,19,23,31-33].

Ethion (0,0,0-0-tetraethyl-5,5'-methylen-bis (phosphorodithioate)) is one of the widely used OPs, which have been identified as contaminants in many component of the global ecosystem. It is used to kill aphids, mites, thrips, leafhoppers and maggots. Previous studies have indicated that ethion exposure caused increase in LPO and significantly altered the activities of the antioxidant enzymes [34,35]. Many other studies have been carried out to study the toxic effects of OPs but data on ethion-induced toxicity is scanty. Since the mechanism of action of ethion has not been fully elucidated, further information on the factors regulating the toxicity of ethion should allow a better assessment of their environmental impact. So the present study was planned to investigate the toxic effects of ethion and ameliorative role of vitamin E in rat model.

2. Material and methods

2.1. Chemicals

Technical grade ethion was a gift from Rallis, India. Vitamin E (α-tocopheryl acetate, trade name Evion) was purchased from the Merck Pharmaceuticals (Mumbai, India). Other chemicals were purchased from Sigma-Aldrich, St. Louis, USA and Sisco Research Laboratory (SRL), Mumbai, India.

2.2. Experimental design

Adult male albino rats of Wistar strain, weighing 120-180 g were purchased from central animal house of Panjab University, Chandigarh. All the animals were housed in clean polypropylene cages and were fed standard diet (Ashirwad Industries, Kharar, India) with free access to water on a 12 h light/dark cycle. All the experiments were performed according to guidelines for use and care of laboratory animals and were approved by the ethical committee of the Panjab University, Chandigarh. The body weight of all the animals was checked regularly. The animals were randomly divided into four groups, each comprising of six animals. Corn oil was used as a vehicle for oral administration of ethion.

Group I (control): Animals were administered corn oil only.
Group II (vitamin E treated): Animals were administered vitamin E (50 mg/kg bw/day).
Group III (ethion treated): Animals were administered ethion (2.7 mg/kg of bw/day) dissolved in corn oil.
Group IV (ethion + vitamin E treated): Animals were administered ethion (2.7 mg/kg of bw/day) along with vitamin E (50 mg/kg bw/day).

The body weight of each rat was taken before sacrifice. The rats were sacrificed on Days 7, 14, 21 or 28 by decapitation under ether anesthesia followed by an overdose of thiopental. Blood and liver tissues collected were then used for various biochemical assays and histopathological examinations. The liver tissue was washed twice with ice cold 0.1 M phosphate buffer saline (1:9), pH 7.4, blotted, dried and weighed. A small portion of the tissue was used for histopathological examinations. The remaining tissue was stored at -20 °C for further analysis. A 10X tissue homogenate was prepared in 50 mM Tris–HCl solution (pH 7.4) by homogenizing the tissue using Potter–Elvehjem glass homogenizer. The homogenate was centrifuged at 6000 g for 15 min at 4 °C to remove the cell debris and then the supernatant obtained was used for the determination of LPO and antioxidant enzymes in control and experimental rats. Acetylcholine esterase (AChE) activity was measured in erythrocytes.

2.3. Biochemical measurements

2.3.1. Measurement of protein content

The protein content in the liver homogenate was estimated according to the method of Lowry et al. [36].

2.3.2. Measurement of malondialdehyde (MDA) level

As an index of lipid peroxidation, the formation of TBARS was measured in liver tissues according to the method of Wills [37]. The amount of malondialdehyde (MDA) formed was measured by the reaction with thiobarbituric acid at 532 nm. The results were expressed as nanomoles MDA per milligram protein using molar extinction coefficient of MDA thiobarbituric chromophore (1.56 × 10^5 M^-1 cm^-1). The results were expressed in terms of the extent of malondialdehyde (MDA) production.

2.3.3. Antioxidant enzymes assay

SOD activity was assayed in the liver tissue according to the method of Kono [38]. Superoxide anions are generated by the oxidation of hydroxylamine hydrochloride. The reduction of nitro blue tetrazolium (NBT) to blue formazan mediated by superoxide anions was measured at 560 nm under aerobic conditions. Addition of superoxide dismutase inhibits the reduction of NBT mediated by hydroxylamine hydrochloride. The extent of inhibition is taken as measure of enzyme activity.

Catalase (CAT) activity was measured in liver homogenate according to the method of Abeij [39]. The principle of the assay is based on the determination of the rate constant of decomposition of hydrogen peroxide by the enzyme catalase.

Glutathione-S-transferase (GST) activity in liver homogenate was assayed by the procedure of Habig et al. [40], using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate which absorbs maximum at 340 nm and have an extinction coefficient of 9.6 mM^-1 cm^-1.

Glutathione reduced (GSH) content were quantified in the liver homogenate according to the method described by Ellman et al. [41]. In this method 5,5'-dithiobis-2-nitrobenzoic acid (DNTB) is reduced by dry–SH groups to form one mole of 2-nitro-S-mercaptopropionic acid per mole of dry-SH. The nitromercaptobenzonic acid released has an intense yellow color and can be used to measure dry-SH groups.

Glutathione reductase (GR) activity in liver homogenate was determined by following the oxidation of NADPH to NADP by the enzyme catalese. The oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance of NADPH was measured at 340 nm wavelength.

Glutathione reductase (GR) activity in liver homogenate was measured by the method of Paglia and Valentine [42]. GSH-Px catalyzes the oxidation of glutathione by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance of NADPH was measured at 340 nm wavelength.

2.3.4. Histopathological examinations

The liver samples were fixed in 10% buffered formalin, processed through graded alcohols and xylene and embedded in paraffin blocks. Tissue sections were cut at 5 μm at multiple levels and
stained with haematoxylin-eosin. Mounted slides were examined and photographed under a photomicroscope. All histological evaluations were made twice under blind conditions (without knowledge of the treatment).

2.4. Statistical analysis

All values were expressed as mean ± SD of six animals per group. Data were analyzed using one way analysis of variance (ANOVA) followed by multiple pair wise comparisons between the various treated groups. Values with p < 0.05 were considered as statistically significant. All the data were analyzed by using SPSS version 14.

3. Results

3.1. Effects of in vivo administration of ethion on body weight of rats

Fig. 1 shows the effect of ethion-induced toxicity on body weight of control and experimental groups. There was a significant decrease in the body weight of rats intoxicated with ethion compared with control and vitamin E treated animals. This decrease in the body weight of ethion-treated rats becomes more pronounced with the increase in the duration of ethion treatment. The weight loss due to ethion toxicity was recovered to near normal values with the supplementation of vitamin E in ethion + vitamin E treated group.

3.2. Effects of in vivo administration of ethion on AChE activity

Table 1 shows the effect of administration of ethion on AChE activity of erythrocytes. A significant decrease in the AChE activity was observed in ethion treated group compared to control. However, supplementation of vitamin E along with ethion resulted in a significant increase in the AChE activity.

3.3. Effects of in vivo administration of ethion on lipid peroxidation

The results are shown in Table 2. The levels of MDA increased significantly in the ethion treated group at all the time intervals of treatment. The differences between the ethion + vitamin E and control groups in terms of the MDA levels were significant (p < 0.05). Vitamin E treated rats showed LPO comparable to control. Treatment with ethion showed increased LPO in liver tissue as compared to control. Ethion + vitamin E administered rats showed significant decrease in LPO as compared to rats administered ethion without vitamin E. Lipid peroxidation was increasing significantly with the duration of the treatment of ethion. A significant (p < 0.001) decrease in the LPO was observed when were treated with vitamin E along with ethion as compared to control and vitamin E treated rats at all the time intervals of treatment. In the lipid peroxidation in the tissue suggests that oxidant is induced by ethion.

3.4. Effects of ethion-induced toxicity on reduced glutathion content

The levels of GSH in the liver tissue of experimental groups presented in Table 3. Comparison of GSH contents in the mental groups showed that GSH content were insignificantly in vitamin E treated group than in the control group administration led to decrease in GSH content in the ethion group. It was seen that there was a significant increase in GSH in ethion + vitamin E administered groups when compared those in the ethion (Table 3). However, the levels of GSH in the ethion and ethion + vitamin E treated rats were significantly lower than the controls and vitamin E treated rats. These indicate that ethion intoxication decreased the GSH content in the liver tissue and administration of vitamin E showed in their GSH content as compared to ethion treated group.

3.5. Effects of ethion intoxication on antioxidant defense system

The activities of antioxidant enzyme such as SOD, CAT, and GR in control and experimental animals were presented in Table 4. Significant increase in the activities of SOD, CAT and GR were observed in ethion treated group when compared with control or vitamin E groups. On simultaneous supplementation with vitamin E to ethion treated group, a significant increase in the activities of SOD, CAT, GPx and GST were observed while compared with ethion alone treated group, which theta the restoration of normalcy to some extent in vitamin E treated group. No significant change in the GR activity was observed control and vitamin E treated rats, but a significant increase in their activity was found after 21 and 28 days of supplementation with vitamin E compared to control group. Treatment with vitamin E markedly inhibited GR activity as compared to control group and vitamin E treated rats, but a significant increase in their activity was found after 21 and 28 days of supplementation with vitamin E compared to control group. Treatment with vitamin E markedly inhibited GR activity as compared to control group and vitamin E treated rats, but a significant increase in their activity was found after 21 and 28 days of supplementation with vitamin E compared to control group. Treatment with vitamin E markedly inhibited GR activity as compared to control group.

3.6. Histopathological findings

Results of histopathological examination of liver show control and vitamin E treated group were within normal. The central part of the liver lobules showing a central...
Table 1
The effect of in vivo administration of ethion and ethion + vitamin E on AChE activity (nmol acetylthiocholine/min/mg protein) in erythrocyte membrane of all groups.

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>77.01 ± 1.80</td>
<td>76.83 ± 4.16</td>
<td>77.28 ± 4.32</td>
<td>77.25 ± 3.28</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>77.39 ± 3.72</td>
<td>77.73 ± 4.32</td>
<td>78.14 ± 4.26</td>
<td>78.74 ± 1.84</td>
</tr>
<tr>
<td>Ethion</td>
<td>56.42 ± 4.37 **</td>
<td>51.68 ± 4.26 *</td>
<td>48.59 ± 4.57 *</td>
<td>45.04 ± 4.15 *</td>
</tr>
<tr>
<td>Ethion + vitamin E</td>
<td>66.05 ± 4.44</td>
<td>60.32 ± 3.80 **</td>
<td>57.60 ± 3.00 **</td>
<td>53.09 ± 3.65 **</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six animals in each group. *p < 0.05 was considered significant.
1. Significantly different from control group (*p < 0.05).
2. Significantly different from vitamin E group (**p < 0.05).
3. Significantly different from ethion group (***p < 0.05).

Table 2
The effects of in vivo administration of ethion and vitamin E supplementation on lipid peroxidation (nmol MDA/mg protein) in liver of all groups.

<table>
<thead>
<tr>
<th>Treatment of animals</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.62 ± 0.08</td>
<td>2.00 ± 0.03</td>
<td>2.59 ± 0.04</td>
<td>2.56 ± 0.04</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>2.21 ± 0.05 *</td>
<td>2.22 ± 0.04 *</td>
<td>2.09 ± 0.03 *</td>
<td>1.88 ± 0.05 *</td>
</tr>
<tr>
<td>Ethion</td>
<td>3.43 ± 0.09 **</td>
<td>4.07 ± 0.08 **</td>
<td>5.77 ± 0.15 **</td>
<td>6.31 ± 0.19 **</td>
</tr>
<tr>
<td>Ethion + vitamin E</td>
<td>2.26 ± 0.05 *</td>
<td>2.85 ± 0.10 *</td>
<td>3.40 ± 0.21 *</td>
<td>4.51 ± 0.16 *</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six animals in each group. *p < 0.05 was considered significant.
1. Significantly different from control group (*p < 0.05).
2. Significantly different from ethion group (**p < 0.05).
3. Significantly different from ethion group (***p < 0.05).

Table 3
The effects of in vivo administration of ethion and ethion + vitamin E on reduced glutathione (GSH) content (nmol GSH/mg protein) in liver tissue of rats.

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73.00 ± 1.5</td>
<td>73.80 ± 2.0</td>
<td>72.90 ± 0.9</td>
<td>73.10 ± 1.0</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>77.30 ± 2.9</td>
<td>78.10 ± 1.2</td>
<td>78.70 ± 2.5</td>
<td>80.80 ± 3.9</td>
</tr>
<tr>
<td>Ethion</td>
<td>53.20 ± 1.3 **</td>
<td>49.10 ± 1.7 **</td>
<td>43.80 ± 1.7 **</td>
<td>38.70 ± 3.7 **</td>
</tr>
<tr>
<td>Ethion + vitamin E</td>
<td>61.40 ± 1.6 *</td>
<td>57.20 ± 2.8 *</td>
<td>51.40 ± 2.2 *</td>
<td>45.40 ± 1.4 *</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six animals in each group. *p < 0.05 was considered significant.
1. Significantly different from control group (*p < 0.05).
2. Significantly different from ethion group (**p < 0.05).
3. Significantly different from ethion group (***p < 0.05).

Organophosphate insecticides are used extensively worldwide, and poisoning by these agents, particularly in developing nations including India, is a serious public health problem [44]. They have been shown to exert deleterious effects on biological systems through the inhibition of AChE activity in erythrocytes. In the present study, in vivo administration of ethion inhibits AChE activity in erythrocytes. An in vitro study on ethion-induced toxicity revealed that ethion inhibited AChE activity in erythrocyte membrane [34]. In another study, AChE was found to be inhibited in plasma and red blood cells of goats after ethion exposure [35]. AChE activity was found to be inhibited after the administration of ethion and fenitrothion to silk worm [45]. Present data shows that supplementation of vitamin E along with ethion might partially restore the activity of AChE in ethion-treated rats. Similar results were shown by previous studies [15,7,19,31]. Matkovics et al. [46] reported that the AChE-inhibiting action of OPIs is better compensated by vitamin E. Sutcu et al. [55] demonstrated that methidathion caused a significant decrease in the AChE activity.

Experimental studies have shown that oxidative stress in biological systems originates as a result of imbalance between the generation of oxidizing species and cellular antioxidant defenses [1,12-14,16-20,22-29,31,47]. Results presented in the present investigation indicate that exposure to ethion increases the LPO level in rat liver tissue. However, supplementation of vitamin E along with the administration of ethion led to a significant decrease in LPO, indicating that it may have beneficial role in lowering ethion-induced toxicity. Singh et al. [34] have shown that ethion caused increased LPO in erythrocytes. Recent studies reported that various organophosphate insecticides caused increase of LPO levels in rat liver [21,22,28,29,48,49]. The increase in MDA might be modulated by ethion itself inducing LPO or by a possible increase in ROS induced by ethion. ROS are part of normal oxidative metabolism, but when produced in excess, they cause tissue injury including lipid peroxidation, DNA damage, and enzyme inactivation [50,51]. In addition, oxidative stress is also a process related to xenobiotic exposure and different levels of environmental contamination [50]. In such cases, peroxidation of membrane lipids seems to be an unavoidable process in tissue injury, and may impair antioxidant defenses, leading to oxidative damage by changing the balance between oxidants and antioxidants [1,30,52]. These results support the hypothesis whereby LPO has been suggested as one of the molecular mechanisms involved in OPI-induced toxicity.

The cells have several ways to alleviate the effects of oxidative stress, either by repairing the damage or by directly diminishing

4. Discussion

Organophosphate pesticides are used extensively worldwide, and poisoning by these agents, particularly in developing nations including India, is a serious public health problem [44]. They have been shown to exert deleterious effects on biological systems through the inhibition of AChE activity in erythrocytes. In the present study, in vivo administration of ethion inhibits AChE activity in erythrocytes. An in vitro study on ethion-induced toxicity revealed that ethion inhibited AChE activity in erythrocyte membrane [34]. In another study, AChE was found to be inhibited in plasma and red blood cells of goats after ethion exposure [35]. AChE activity was found to be inhibited after the administration of ethion and fenitrothion to silk worm [45]. Present data shows that supplementation of vitamin E along with ethion might partially restore the activity of AChE in ethion-treated rats. Similar results were shown by previous studies [15,7,19,31]. Matkovics et al. [46] reported that the AChE-inhibiting action of OPIs is better compensated by vitamin E. Sutcu et al. [55] demonstrated that methidathion caused a significant decrease in the AChE activity.

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The cells have several ways to alleviate the effects of oxidative stress, either by repairing the damage or by directly diminishing
Table 4
The effects of in vivo administration of ethion and ethion + vitamin E on antioxidant enzymes in liver tissue (U/mg protein) of all groups.

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>7 Days</th>
<th>14 Days</th>
<th>21 Days</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD activity (U/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>34.80 ± 2.62</td>
<td>34.77 ± 1.96</td>
<td>35.53 ± 1.12</td>
<td>34.21 ± 1.96</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>26.48 ± 1.57</td>
<td>26.01 ± 1.67</td>
<td>25.80 ± 2.03</td>
<td>25.54 ± 1.96</td>
</tr>
<tr>
<td>Ethion</td>
<td>43.33 ± 1.43 *</td>
<td>48.51 ± 1.25 *</td>
<td>57.62 ± 1.50 *</td>
<td>64.51 ± 1.43 *</td>
</tr>
<tr>
<td>Ethion + vitamin E</td>
<td>38.23 ± 1.46 **</td>
<td>41.89 ± 1.63 **</td>
<td>48.13 ± 1.10 **</td>
<td>55.14 ± 1.46 **</td>
</tr>
<tr>
<td>Catalase activity (μmol H₂O₂ decomposed/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>83.06 ± 3.85</td>
<td>85.19 ± 6.63</td>
<td>85.09 ± 5.80</td>
<td>85.72 ± 6.53</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>81.99 ± 4.37</td>
<td>80.11 ± 5.45</td>
<td>77.56 ± 4.83</td>
<td>74.02 ± 5.37</td>
</tr>
<tr>
<td>Ethion</td>
<td>94.27 ± 4.37 *</td>
<td>109.85 ± 5.22 *</td>
<td>117.08 ± 4.83 *</td>
<td>127.81 ± 5.37 *</td>
</tr>
<tr>
<td>Ethion + vitamin E</td>
<td>87.14 ± 4.63</td>
<td>92.19 ± 3.10 **</td>
<td>102.70 ± 7.17 **</td>
<td>115.60 ± 5.37 **</td>
</tr>
<tr>
<td>GSH-Px activity (nmol NADPH oxidized/min/mg protein)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>74.26 ± 4.56</td>
<td>75.83 ± 3.91</td>
<td>75.07 ± 2.85</td>
<td>74.35 ± 3.91</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>73.58 ± 3.36</td>
<td>75.40 ± 4.55</td>
<td>75.71 ± 3.94</td>
<td>76.51 ± 3.36</td>
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<tr>
<td>Ethion</td>
<td>86.18 ± 5.75 *</td>
<td>112.04 ± 4.63 **</td>
<td>190.04 ± 5.93 *</td>
<td>219.51 ± 5.75 *</td>
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<tr>
<td>Ethion + vitamin E</td>
<td>76.66 ± 5.21</td>
<td>87.02 ± 3.94 **</td>
<td>120.78 ± 8.00 **</td>
<td>169.84 ± 5.21 **</td>
</tr>
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<td>GST activity (nmol CDNB conjugated/min/mg protein)</td>
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<td></td>
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<tr>
<td>Control</td>
<td>424.4 ± 7.90</td>
<td>421.6 ± 12.10</td>
<td>425.70 ± 10.30</td>
<td>4205.6 ± 7.90</td>
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<tr>
<td>Vitamin E</td>
<td>420.60 ± 8.99</td>
<td>417.20 ± 6.66</td>
<td>412.70 ± 8.90</td>
<td>407.14 ± 8.99</td>
</tr>
<tr>
<td>Ethion</td>
<td>482.50 ± 11.27 **</td>
<td>563.10 ± 15.57 **</td>
<td>740.90 ± 14.97 **</td>
<td>528.71 ± 11.27 **</td>
</tr>
<tr>
<td>Ethion + vitamin E</td>
<td>452.30 ± 11.77 **</td>
<td>474.70 ± 11.11 **</td>
<td>506.20 ± 9.26 **</td>
<td>579.80 ± 11.77 **</td>
</tr>
<tr>
<td>GR activity (nmol of NADPH oxidized/min/mg protein)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>77.20 ± 2.51</td>
<td>77.82 ± 1.38</td>
<td>76.91 ± 2.61</td>
<td>78.81 ± 2.51</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>82.98 ± 2.26</td>
<td>83.88 ± 2.89</td>
<td>85.72 ± 2.58</td>
<td>86.51 ± 2.26</td>
</tr>
<tr>
<td>Ethion</td>
<td>65.79 ± 2.71 *</td>
<td>61.25 ± 2.21 **</td>
<td>54.10 ± 1.84 *</td>
<td>44.41 ± 2.71 **</td>
</tr>
<tr>
<td>Ethion + vitamin E</td>
<td>72.61 ± 1.95</td>
<td>68.24 ± 2.05 **</td>
<td>63.18 ± 1.88 *</td>
<td>56.71 ± 1.95 **</td>
</tr>
</tbody>
</table>

Values are mean ± SD of six animals in each group, *p < 0.05 was considered significant.
in terminating free radical production due to ethion-induced toxicity. This enhanced intracellular transport of GSH seems to be essential in maintaining the redox state and to cope with the oxidative stress in the vitamin E treated rats. Various OPs such as chlorpyri fos-ethyl, dimethoate, malathion and atrazine have been reported to decrease the GSH level both in vivo and in vitro in rats [54, 57– 59]. It has been reported that GSH play important role in protecting cells from xenobiotics induced tissue injury [60, 61]. The decreased hepatic GSH content in ethion-treated rats may probably be due to increased activity of GST in liver. The increase in GST activity may increase the conjugation of -SH groups thus protecting the liver from ethion-induced toxicity. GST detoxicates a variety of electrophilic compounds to less toxic forms by conjugation with -SH groups such as GSH. The marked increase in GST activity in ethion-treated rats indicates sufficient conjugation of electrophiles and detoxication of these species. Sharma et al. [22] have reported increase in the activity of hepatic GST after dimethoate administration. However, the recovery in liver GST activity in vitamin E along with ethion-treated rats may be due to lower oxidative stress. Recent studies have suggested that vitamin E plays a protective role against the oxidative stress induced by various OPs [19, 20, 24, 25, 55, 62]. Glutathione reductase (GR) activity in liver tissue was significantly decreased in ethion-treated rats, suggesting an inadequate level of NADPH and a failure to maintain GSH levels. The decrease in enzyme activity also suggests the possible free radical mediated oxidative stress and consequent damage to liver. 1 activity of GR was slightly increased in simultaneous adminis- 

Present study reported that ethion induces the histopathological changes in the liver. There are evidences for histopathological changes in liver of OPs treated animals through induction of toxic liver tissue stress [20, 22, 25, 32, 63]. These histopathological alterations could be prevented by administration of antioxidants like vitamin E a C. Gokalp et al. [63] showed that acute MD treatment caused a significant increase in histopathological changes in the liver tissue. Additionally, treatment with vitamins E and C led to significant increase in the histopathological changes. Sutcu et al. [25] demonstrated histopathological changes in liver tissue of rats intoxicated with methidathion.

In conclusion, these findings demonstrate that in vivo chronic administration of ethion resulted in histopathological modifications and oxidative stress indicated by increase in lipid peroxidation and changes the activities of antioxidant enzyme in rat liver. Supplementation of vitamins E may ameliorate toxic effects of ethion. The protective effects of natural and synthetic antioxidants against ethion-induced histopathological and biochemical alterations are the most important evidences involvement of oxidative stress in ethion-induced toxicity.

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References


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Dear Dr. Sandhir,

In the light of the comments from the reviewers for your manuscript, I am pleased to inform you that the revised manuscript of your research paper entitled, ‘Biochemical and Morphological Perturbations in Rat Erythrocytes Exposed to Ethion: Protective Effect of Vitamin E’ submitted for publication in Cellular and Molecular Biology has been accepted.

Thanking you,

Sincerely yours,

(Bechan Sharma)