



## Ameliorative Effect of Vitamin E on Cypermethrin Induced Hepatotoxicity and Oxidative Stress in Male Wistar Rats

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### ABSTRACT

The present study was carried out to investigate the ameliorative effect of vitamin E on Cypermethrin induced hepatotoxicity and oxidative stress. For this purpose, a total of 24 rats were randomly divided into four equal groups: group I used as control and groups II, III and IV were orally treated with Cypermethrin (25 mg/kg body weight), Vitamin E (100 mg/kg body weight) and Cypermethrin (25 mg/kg body weight) plus Vitamin E (100mg/kg body weight), respectively for 45 days. Results showed that on administration of Cypermethrin the mean values of superoxide dismutase (SOD) decreased significantly. Similarly the mean values of reduced glutathione (GSH) and catalase were significantly decreased, while there was significant increase in the mean value of lipid peroxidation (LPO) in liver homogenate. Enzymatic activities of aspartate aminotransferase (AST), alanine (ALT) and alkaline phosphatase (ALP) in plasma were significantly increased due to Cypermethrin administration. Further, light microscope investigation revealed that Cypermethrin exposure induced histopathological alterations in the liver tissue. On the other hand; treatment with Vitamin E alleviated the harmful effect of Cypermethrin in the group (Group IV). Thus, present study revealed that the presence of Vitamin E could diminish the hepatotoxicity and oxidative stress in male wistar rats.

**Keywords:** Hepatotoxicity, cypermethrin, oxidative damage, vitamin E, rats

Cypermethrin (CY) is a highly active synthetic pyrethroid insecticide and most widely used to control pests and is frequently detected from vegetables and fruits. Cypermethrin with potent insecticidal property mainly acts as a stomach and contact insecticide (Jin and Webster, 1998). Environmental contaminations due to pyrethroids have been reported for increasing oxidative stress in mammals thereby producing morbid lesion (Daniel and Moser, 1993; Yousef *et al.*, 2003).

Vitamin E is a naturally occurring antioxidant nutrient, and a lipid-soluble vitamin present in lipid bilayer membranes that plays important role in animal health by inactivating harmful free radicals and inhibits free radical formation (Kalender *et al.*, 2004). Vitamin E is known for its antioxidant property protecting the unsaturated bonds of phospholipids present in the cell membrane against free radical damage (Burton *et al.*, 1986). The present study deals with the hepatotoxicity and oxidative stress of

Cypermethrin and the ameliorative effect of vitamin E in wistar rats.

### MATERIALS AND METHODS

All the experimental procedures, housing and management of the rats were strictly carried out according to the recommendations and approval of the Institutional Animal Ethics Committee (IAEC) as per the guidelines set forth by committee for the purpose of control and supervision of experiments on animals (CPCSEA). The test chemical Cypermethrin (Cypermethrin technical, Batch no. CMN920T7205B, purity 92% by mass) was obtained from Gharda Chemicals Limited, Ratnagiri, Maharashtra (India), whereas Vitamin E acetate ( $C_{31}H_{52}O_3$ , CAS no. 7695-91-2, purity- 95%) used in this study was procured from Central drug house, New Delhi. All other chemicals used were standard analytical grade chemicals and test kits were procured from SRL (India), Merk (India),

HiMedia (India), BDH, Qualigens, Span diagnostic Ltd. (India) and CDH (India). Twenty four healthymale Wistar rats (4 weeks old) weighing between 80-100 grams were procured from Laboratory Animal Resources (LAR) Section of Indian Veterinary Research Institute (IVRI), Izatnagar (U.P.). Adequate lighting (12 hours light and 12 hour darkness), ventilation, temperature ( $21\pm 2^{\circ}\text{C}$ ), relative humidity ( $50\pm 10\%$ ) and hygienic conditions were maintained throughout the experiment. The rats were provided with paddy husk as bedding material in the cages which was changed thrice a week to keep the surroundings dry. The animals were maintained under standard managemental conditions and provided feed and water *adlibitum*. All the rats were given standard diet procured from Ashirwad Industries Limited, Punjab. After 15 days of acclimation, the rats were randomly divided into four equal groups, each group containing 6 rats: group I used as control (received normal water) and groups II, III and IV were orally treated with Cypermethrin (25 mg/kg body wt in corn oil), Vitamin E (100 mg/kg body weight in corn oil) and Cypermethrin plus Vitamin E (in corn oil), respectively for 45 days. The experiment continued for 45 days. The clinical symptoms were observed daily in each group while biochemical parameters, parameters of oxidative stress and pathomorphological studies were carried out on day 45 of experiment. The blood samples were collected from retro-orbital plexus on day 45 from the rats of all the groups using micro-capillary tubes in 5.0 ml vacutainer containing heparin as anticoagulant. Heparinised blood samples were centrifuged at 2000 rpm for 15 min. Plasma was separated and stored at  $-20^{\circ}\text{C}$  for further analysis of biochemical anylates. The biochemical parameter studied were aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in the rats of different groups using standard diagnostic kits (Span Diagnostic Ltd., Surat).

Rats belong to all the groups were sacrificed on day 45, the termination of experiment. Feed was withdrawn 24 hr before sacrifice. Liver was collected, washed with ice cold normal saline and weighed and stored at  $-80^{\circ}\text{C}$  until assayed. Estimation of different oxidative stress parameters viz. lipid peroxidation (LPO), superoxide dismutase (SOD), reduced glutathione and catalase in liver were carried out by double beam UV-VIS spectrophotometer.

Frozen liver samples were thawed at room temperature and 200 mg of sample was weighed and taken in 2 ml of

ice-cold saline for estimation of LPO, SOD and Catalase. An amount of 200 mg of sample was weighed separately and taken in 2 ml of 0.02 M EDTA (ethylene diamine tetra acetic acid) for GSH estimation. The homogenates prepared by using homogenizer, under cold conditions were centrifuged for 10 min at 3000 rpm. The supernatant was used for assay of glutathione, lipid Peroxidation, superoxide dismutase and Catalase.

The extent of lipid peroxidation was evaluated in terms of MDA (malondialdehyde) production, determined by thiobarbituric acid (Rehman, 1984). GSH was determined by estimating free -SH groups, using DTNB method of (Sedlak and Lindsay, 1968). Superoxide SOD was estimated as per the method described by (Madesh and Balasubramanian, 1998) whereas catalase was assayed and calculated in tissue homogenate as for the method prescribed by (Bergmeyer, 1983).

The quantitative data of biochemical observations as well as oxidative stress parameters were analyzed by Duncan's multiple range tests as per (Snedecor and Cochran, 1989).

## RESULTS AND DISCUSSION

Liver is a prime organ associated with xenobiotic metabolism (Hinton and Grasso, 2000). Perhaps, production of metabolically toxic intermediates capable of causing hepatocellular damage during the detoxification of Cypermethrin in liver, causing respective leakage of these enzymes in the blood (Ahmed *et al.*, 2011; Bhushan *et al.*, 2013). However, antioxidants like Vitamin E can counteract/reduce this toxicity (Sharaf *et al.*, 2010). Therefore to test the protective effect of Vitamin E on liver damage caused by Cypermethrin, an experiment was conducted on-24 wistar rats as described earlier in this paper.

The results of biochemical parameters are summarized and presented in Table 1. Briefly, the mean values of AST, ALT and ALP revealed significant ( $P < 0.01$ ) increase in the rats of groups-II and group-IV as compared to the rats of group-I and group-III. There was also significant variation in the mean values of AST, ALT and ALP in the rats of group-II as compared to group-IV. Non-significant variation was observed in the mean values of AST, ALT and ALP in rats of groups-III as compared to the rats of group-I on day 45 of experiment.

**Table 1:** Changes in values of different biochemical parameters on day 45 in Wistar rats. (mean  $\pm$  SEM, N=6)

Parameters	Group I	Group II	Group III	Group IV
AST(IU/L)	68.25 <sup>A</sup> $\pm$ 0.26	122.18 <sup>C</sup> $\pm$ 1.12	66.71 <sup>A</sup> $\pm$ 0.19	104.66 <sup>B</sup> $\pm$ 0.86
ALT(IU/L)	37.64 <sup>A</sup> $\pm$ 0.18	68.14 <sup>C</sup> $\pm$ 0.32	37.15 <sup>A</sup> $\pm$ 0.46	53.99 <sup>B</sup> $\pm$ 0.74
ALP(IU/L)	42.23 <sup>A</sup> $\pm$ 1.0	60.74 <sup>C</sup> $\pm$ 0.36	41.37 <sup>A</sup> $\pm$ 0.85	52.36 <sup>B</sup> $\pm$ 0.53

Mean with different superscript (A, B, C) differing significantly in between the groups, otherwise non-significant either row or column wise?

**Table 2:** Mean values of different oxidative stress parameters in liver tissue in different experimental groups on day 45 in Wistar rats (mean  $\pm$  SEM, N=6)

Group	LPO (nM MDA/g tissue)	GSH (mMGS/g tissue)	SOD (U/ mg of protein)	CAT (mMH <sub>2</sub> O <sub>2</sub> utilized/min/ mg of protein)
I	68.19 <sup>A</sup> $\pm$ 0.57	2.51 <sup>A</sup> $\pm$ 0.03	22.16 <sup>A</sup> $\pm$ 0.58	87.11 <sup>A</sup> $\pm$ 0.82
II	81.10 <sup>C</sup> $\pm$ 0.59	1.90 <sup>B</sup> $\pm$ 0.05	16.09 <sup>B</sup> $\pm$ 0.56	70.11 <sup>B</sup> $\pm$ 1.49
III	67.14 <sup>A</sup> $\pm$ 0.58	2.75 <sup>A</sup> $\pm$ 0.04	23.06 <sup>A</sup> $\pm$ 0.58	90.13 <sup>A</sup> $\pm$ 0.80
IV	73.09 <sup>B</sup> $\pm$ 0.57	2.2 <sup>B</sup> $\pm$ 0.05	20.12 <sup>BC</sup> $\pm$ 0.57	78.13 <sup>B</sup> $\pm$ 0.80

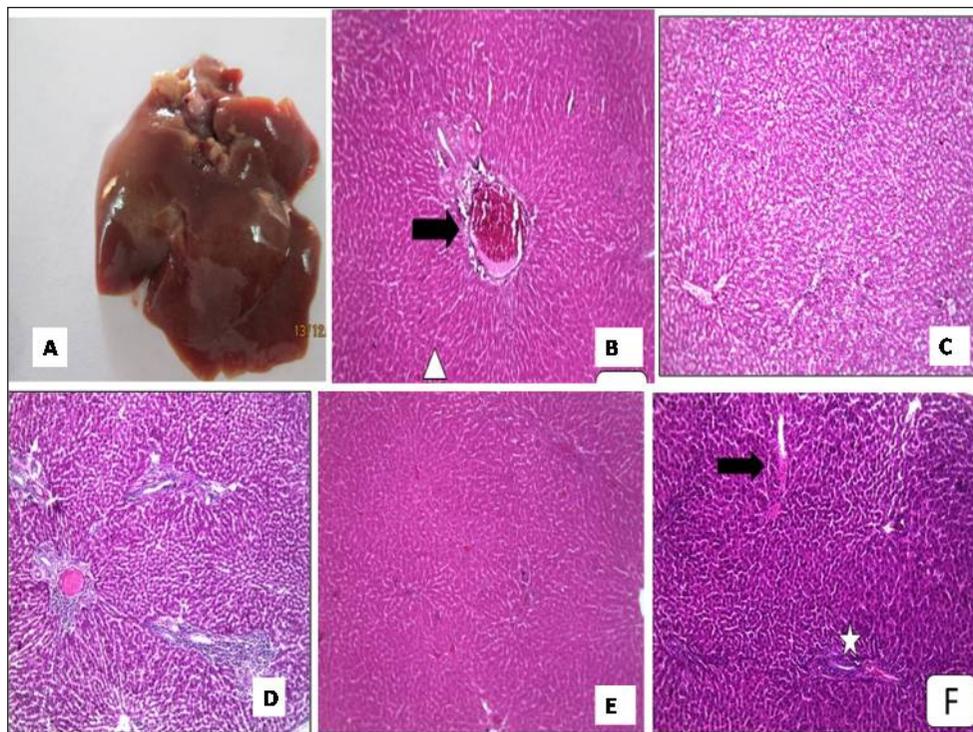
Mean with different superscript (A, B, C) differing significantly in between the groups, otherwise non-significant.

Enzymes like AST and ALT represent the functional status of the liver. In the present study, the rats of group-II showed elevations in AST, ALT and ALP activity from the exposure of the rats to the Cypermethrin. ALT activity is related to general hepatocellular dysfunction and AST to mitochondrial damage. Increased aminotransferase (AST and ALT) activity in serum reflects hepatocellular damage leading to leakage of these enzymes into general circulation (Sharaf *et al.*, 2010; Bhushan *et al.*, 2013). The increases in level of ALT, AST and ALP in Cypermethrin toxicity are in accordance with the findings in the albino rats (Manna *et al.*, 2004; Bhushan *et al.*, 2013). In contrast to the present study, Kaur and Sandhu (2000) observed a significant decrease in the plasma AST and ALT activities in buffalo calves when 0.1% of Cypermethrin was sprayed dermally for 10 consecutive days. Cypermethrin is capable of altering normal hepatocellular architecture (Yavasoglu *et al.*, 2006; Bhushan *et al.*, 2013). Therefore, increase in activity of serum alkaline phosphatase (ALP) in the present study can be attributed initially to some patho-physiological changes in liver as a consequence of pesticide intoxication or may be due to damage in membrane permeability of hepatocytes, resulting in leakage of this enzyme into the blood stream.

The mean changes of oxidative stress in liver tissue are summarized in Table 2. The mean values of LPO in liver

tissue revealed significant ( $P < 0.01$ ) increase and the mean values of GSH, SOD and Catalase showed significant ( $P < 0.01$ ) decrease in the rats of groups-II and group-IV as compared to the rats of group-I and group-III. There was also significant variation in the mean values of LPO, GSH, SOD and Catalase in the rats of group-II as compared to the rats of group-IV. GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. Under oxidative stress, GSH is consumed by GSH related enzymes (GPx and GST) to detoxify the peroxides produced due to increased lipid peroxidation (Hayes *et al.*, 2005). A pronounced decrease of GSH level was found in liver of the rats intoxicated with Cypermethrin. This may be responsible for enhancement of lipid peroxidation. Lower activities of GSH levels suggest that Cypermethrin toxicity might induce the accumulation of free radicals, consumption of the antioxidants, and production of oxidative stress marked by LPO. The findings of the present study are in accordance with the observation of other worker who reported that increase in the LPO production and decreased GSH level in Cypermethrin induced oxidative stress in Wister rats (Raina *et al.*, 2010; Sankar *et al.*, 2012) and in mice (Rehman *et al.*, 2006).

Inhibition of P450 contents by Cypermethrin may cause oxidative stress resulting in decrease the activities of



**Fig. 1:** **A:** Liver showing paleness and mottling on the dorsal surface in the rat of Cypermethrin toxicity group (II). **B.** Liver showing congestion of central vein (Black solid arrow) with necrosis of hepatocytes (White blank triangle) in the rat Cypermethrin toxicity group (II) (H&E 100X). **C.** Liver showing vacuolization in hepatocytes (White blank arrow) in the rat Cypermethrin toxicity group (II) (H&E 100X). **D.** Liver showing infiltration of large number of mononuclear cells in the portal areas (Solid black star) in the rat of Cypermethrin toxicity group (II) (H&E 100X). **E.** Liver showing mild congestion of central vein in the rat of Vitamin E treated group (IV) (H&E 100X). **F.** Liver showing mild congestion (Black arrow) and infiltration of mononuclear cells in the portal areas (Blank white star) in the rat of Vitamin E treated group (IV). (H&E 100X).

SOD and catalase (Manna *et al.*, 2004). Decreased SOD levels indicate increased utilization of this enzyme for dismutation of excessive superoxide radicals produced due to Cypermethrin toxicity as well as Catalase is responsible for breakdown of hydrogen peroxide, an important reactive oxygen species produced during metabolism stress conditions (Hertwig *et al.*, 1992). Thus decreased SOD and catalase activities suggests that the finding might be responsible for increased lipid peroxidation following Cypermethrin treatment.

In this study, all the 6 rats of each group were sacrificed on day 45 of experimentation. Grossly, the liver of Cypermethrin toxicity group (II) was pale with occasional presence of pinpoint haemorrhages and mottling on the dorsal surface (Fig. 1A). The liver of Vitamin E treated

ameliorative rats group -IV was slightly pale as compared to rats of groups-II. The rats of control group-I and rats of group-III did not show distinct morbid lesions in liver on 45 days of post administration. Histopathologically, the liver of rats of Cypermethrin intoxicated group-II showed congested sinusoids, central vein and the vessels located in portal areas (Fig. 2B). The hepatocytes showed degenerative changes ranging from cellular swelling to mild to moderate vacuolization (Fig. 2C) with infiltration of large number of mononuclear cells in the portal area (Fig. 2D). Similar microscopic picture were observed in the rats of group-IV ( Fig. 2E and 2F), but of mild in nature as compared to the rats of group-II. The rats of control group-I and rats of group-III did not show distinct morbid lesions in liver on 45 days of post administration (Fig. 1A).

Change in biochemical activities and free radical induced oxidative damage were in accordance with pathomorphological change in the liver of rats intoxicated with Cypermethrin. The observed abnormalities in the liver indicate that Cypermethrin induces toxic effect on liver tissue. Transaminases (AST and ALT) enzymes are a common mean of detecting liver damaging. The alterations in transaminases (AST and ALT) could be associated with pathology involving necrosis of liver. Similar pathological finding were observed in rats in Cypermethrin intoxication (Nair *et al.*, 2011; Bhushan *et al.*, 2013).

As found in this study, supplementation of vitamin E successfully prevented significant changes from Cypermethrin to the activity of ALT, AST, ALP, oxidative stress enzymes and pathological changes. To sum up, the observed protective effect of vitamin E results result from the fact Vitamin E is a naturally occurring antioxidant nutrient, and a lipid-soluble vitamin present in lipid bilayer membranes that plays important role in animal health by inactivating harmful free radicals and inhibits free radical formation (Feher *et al.*, 2007; Kline *et al.*, 2007). Vitamin E is known for its antioxidant property protecting the unsaturated bonds of phospholipids present in the cell membrane against free radical damage (Kamal, 1996). Vitamin E influences the activity of enzymes such as protein kinase C and phospholipase A2 (Bartosz, 2004). Suppression of kinase C and phospholipase in inflammatory cells substantially diminishes the production of free radicals and their effects (Schneider, 2005).

To conclude with it is inferred that the Cypermethrin, a potent nonmetallic compound produced pathological changes in the liver. Liver appeared to be target organ due to toxic effects of Cypermethrin. Administration of vitamin E appeared highly effective as an antioxidant to minimize the Cypermethrin induced hepatotoxicity and oxidative stress in male wistar rats.

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