ABSTRACT
Cypermethrin, a type II synthetic pyrethroid, is widely used in agricultural field, animal husbandry and public health sector. This study was conducted to investigate the effects of cypermethrin on antioxidant enzyme activities and lipid peroxidation (LPO) in female rats and the protective effect of zinc and α-lipoic acid. In this study, cypermethrin was orally administered at 34.33 and 51.5 mg/kg dose levels for consecutive 14 days alone or along with pre-administration of zinc (1-2mg/kg body wt) and α-lipoic acid (35 mg/kg body wt). Cypermethrin caused significant increase in LPO and decrease in reduced glutathione (GSH) level, superoxide dismutase, and glutathione peroxidase activity. Co-administration of zinc and α-lipoic acid attenuated cypermethrin induced ovarian oxidative stress by decreasing LPO in ovary. In addition to this, zinc and α-lipoic acid increased ovarian GSH level and superoxide dismutase, glutathione peroxidase activity. From the findings, it can be concluded that cypermethrin induced oxidative stress in female rat and the administration of zinc and α-lipoic acid provided significant protection against cypermethrin-induced damage due to oxidative stress.

KEYWORDS: Cypermethrin; Oxidative stress; Female rat ovary; Zinc; α-lipoic acid.

INTRODUCTION
Pesticides are being extensively used in agriculture and public health sector to control insects, weeds, and vectors of disease.[1] Pesticides may cause toxicity through several different mechanisms. They exert direct damage to cell structure; interfere with biochemical processes necessary for normal cell functions and causes biotransformation resulting in toxic metabolites.[2] Synthetic pyrethroids are used preferentially in place of organochlorines because they are biodegradable.[3] Cypermethrin, a synthetic pyrethroid is a broad spectrum insecticide and fast acting neurotoxin.[4] Several studies have shown that cypermethrin damages the brain, liver, sperm and erythrocytes by causing oxidative stress.[5] Also, other studies showed that cypermethrin causes free radicals-mediated tissue damages. Zinc, an essential trace element, influences vital processes including cell proliferation, immune function and defense against free radicals.[6][7][8] It activates antioxidant system that prevents cell damage due to oxidative stress.[5][6][8] Zinc is incorporated in oxidant defense system and functions at many levels (Sato and Brenner, 1993).[11] The antioxidant property of zinc is thought to be through maintaining an adequate level of metallothionein and it is essential component of Cu/Zn superoxide dismutase (SOD).[12][13] Alpha-lipoic acid (LA) has become a common ingredient in multivitamin formulas, anti-aging supplements, and even in pet food.

Information regarding the effects of cypermethrin in female rats is scarce and not well defined. Therefore, the present study was undertaken to investigate the effects of cypermethrin on oxidative stress parameters in female albino rats and to investigate the protective effect of zinc and α-lipoic acid on ovarian antioxidant enzyme activities and lipid peroxidation (LPO).

MATERIALS AND METHODS
Chemicals and reagents
Cypermethrin 10% Emulsifiable Concentrate (EC) commercial name (Ustad), sulfosalicylic acid, trichloroacetic acid (TCA), hydrochloric acid (HCl), Tris HCl, 5,5’-dithiobis-(2-nitrobenzoic acid) (DTNB), potassium dihydrogen phosphate (KH₂PO₄), H₂O₂,GSH, CDNB, Sodium dodecyl sulfate, n-Butanol-pyridine, acetate buffer, 2-vinylpyridine, sodium azide, GSSG, NADPH, zinc sulphate (ZnSO₄), were used in the present...
study. All chemicals used were of analytical grade and procured from Sigma-Aldrich, St. Louis, MO, USA; Merck India Ltd., Mumbai, India and Himedia India Ltd., Mumbai, India, SRL Pvt. Ltd., Mumbai, India.

Animal care and treatment
Thirty six Wistar mature female rats were acclimatized for 7 days before the start of the experimental procedure. The animals were housed in labelled cages with solid plastic sides and stainless-steel grid tops and floors, in a room designed for control of temperature (approximately 25±2°C), and light cycle (12 h light, 12 h dark). Animals were fed a standard laboratory pellets diet and water ad libitum. This study was approved by the Institutional Animal Ethical Committee (IAEC), now registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India and done according to the relevant laws and guidelines of the CPCSEA. After 7 days of acclimatization, the animals were randomly assigned to both the experimental groups and the control group, each containing six rats. Groups were designed as:
1. **Group I**: Control (5 ml/kg body wt.)
2. **Group II**: Zinc (1-2 mg/kg body wt) and α-lipoic acid (35 mg/kg body wt.) control
3. **Group III**: Cypermethrin-treated (34.33 mg/kg body wt., Low dose) group
4. **Group IV**: Zn + α-lipoic acid + Cypermethrin-treated (Low dose, 34.33 mg/kg body wt.) group
5. **Group V**: Cypermethrin-treated (51.5 mg/kg body wt., High dose) group
6. **Group VI**: Zinc + α-lipoic acid + Cypermethrin-treated (High dose, 51.5 mg/kg body wt.) group

A commercial formulation of cypermethrin 10% emulsifiable concentrate (EC) was used in the experiments. It was in the form of emulsion and adequate dilutions were done in distilled water in order to get test concentrations (34.34 and 51.5 mg/kg body wt.). The test concentration of cypermethrin was calculated from the percentage of the active ingredient of commercial cypermethrin formulation. Solutions were freshly prepared immediately before experimental administration. On the basis of the toxicity profile of alpha cypermethrin(14), the dose (309 mg/kg bw) of the present study was selected as acute oral LD₅₀ of female rat (albino). The 1/9th LD₅₀ and 1/6th LD₅₀ doses were considered for our experiments. Control rats received 5 ml of distilled water/kg body weight. Body weights of the rats in each group were taken before and after the treatment period. All rats were euthanized 24 h after the last dose. After sacrifice, ovaries were collected from control and treated rats were immediately stored at -20°C.

Estimation of ovarian oxidative stress parameters
Ovarian lipid peroxidation
Ovarian malondialdehyde (MDA) assay was measured by the method of (Ohkawa et al., 1979).(15) One ml tissue homogenate was mixed with 8.1% sodium dodecyl sulfate, acetate buffer (20% pH 3.5), and 1.5 ml of thioarbituric acid (0.8%). After heating at 95°C for 60 min, the red pigment produced was extracted with 5 ml n-butanol-pyridine mixture (15: 1) and centrifuged at 5000 rpm for 10 min at room temperature. The absorbance of supernatants was noted at 535nm.

Ovarian catalase (CAT)
Catalase was estimated by the method of Aebi.(16) The reaction mixture was made up of H₂O₂, double distilled water and 40μl of homogenate (0.05M trishCl). After mixing, readings were noted at 240nm at 30 sec interval.

Ovarian glutathione peroxidase (GPX)
Peroxidase activity was determined by the method (Rotruck et al 1973).(17) 2.5 mM H₂O₂, 0.4 M sodium phosphate buffer, 10mM sodium azide and reduced glutathione was added to tissue homogenate and volume made up to 2 ml with distilled water. It was incubated for 5 min at 37°C and 10% TCA was mixed with the reaction mixture and then centrifuged and the supernatant was mixed with DTNB and Na₂HPO₄. Reading was taken at 412 nm against blank.

Ovarian superoxide dismutase (SOD)
Superoxide dismutase was measured by the method of Marklund and Marklund.(18) At first in a spectrophotometric cuvette, 50 Mn TrisHCl, 10 mM pyrogallol in the presence of EDTA and 20 μl of homogenate were poured and the reading was measured in the spectrophotometer at 420 nm for 3 min.

Ovarian reduced glutathione (GSH)
At first 200μl sample mixed with 100 μl sulfosalicylic acid and centrifuged for 10 min at 3000 rpm. The supernatant was added with 1.8ml of DTNB and was shaken well. Reading was taken at 412-420nm.(19)

Ovarian glutathione-s-transferase (GST)
From ovarian tissue samples of ovarian glutathione-S-transferase were estimated spectrophotometrically(20) using 1-chloro 2,4-dinitrobenzene as substrate. The assay mixture consisted of 1mM CDBN in ethanol,1 M reduced glutathione, 100 mM potassium phosphate buffer (pH-6.5) and supernatant of tissue homogenate. The formed adduct of CDBN, S-2,4-dinitrophenylglutathione was monitored by measuring absorbance at 340 nm against blank.

RESULTS
Figure 1 shows the effect of zinc and α-lipoic acid on ovarian malondialdehyde (MDA) in cypermethrin induced female albino rat. The ovarian MDA level increased significantly (p<0.05, p<0.001) in animals of cypermethrin-treated groups in a dose dependent manner.

As shown in Figure 2, significant decrease (p<0.001) in catalase (CAT) was observed in female rats after cypermethrin-treatment compared to animals of control group. Co-administration of zinc with α-lipoic acid
modified significantly the level of CAT towards normal levels in the treated group animals.

The activities of glutathione peroxidase (figure 3) in the cypermethrin treated group animals were significantly decreased. However, pretreatment with zinc and α-lipoic acid improved glutathione peroxidase activity more or less to the normal status.

Activity of ovarian SOD (Figure 4) was significantly decreased in cypermethrin intoxicated group animals compared to animals of control group. Zinc and α-lipoic acid administrated in cypermethrin-treated rats enhanced significantly the activity of SOD compared to the control animals.

A. Oxidative stress parameters
1) Effect on ovarian malondialdehyde

From figure 5, it is observed that GSH were decreased significantly in cypermethrin treated groups compared to the control rats. Ovarian GSH conten has been decreased significantly (p<0.001) in high dose cypermethrin-treated group and pretreatment with zinc and α-lipoic acid increased GSH level and showed the ameliorating effect on cypermethrin toxicity.

GST level was declined significantly in case of cypermethrin treated group animals compared to control group animals. The present results showed that cypermethrin causes a significant (p<0.001) decrease in the activity of GST in ovarian tissue and it was modified when pretreatment of zinc and α-lipoic acid were done (figure 6).

Figure 1 Shows the effect of zinc and α-lipoic acid on ovarian malon-di-aldehyde in cypermethrin induced female albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups. Asterisks represents the different level of significance (* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001).

2) Effect on ovarian catalase

Figure 2 Illustrates the effect of zinc and α-lipoic acid on ovarian catalase in cypermethrin induced female albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (** indicates p<0.01; *** indicates p<0.001).
3) Effect on ovarian glutathione peroxidase

Figure 3 Shows the effect of zinc and α-lipoic acid on glutathione peroxidase in cypermethrin induced female albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (* indicates p<0.05,** indicates p<0.01, *** indicates p<0.001).

4) Effect on ovarian superoxide dismutase

Figure 4 Illustrates the effect of zinc and α-lipoic acid on superoxide dismutase in cypermethrin induced female albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (* indicates p<0.05,** indicates p<0.01, *** indicates p<0.001).
5) Effect on ovarian reduced glutathione

Figure 5 Shows the effect of zinc and α- lipoic acid on reduced glutathione in cypermethrin induced female albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001).

6) Effect on ovarian glutathione-S-transferase

Figure Illustrates the effect of zinc and α- lipoic acid on glutathione -S-transferase in cypermethrin induced female albino rat. Results are expressed as Mean±SEM. Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Superscript a, Group-I versus all other groups; Superscript c Group-V versus Group-VI. Asterisks represents the different level of significance (* indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001).

DISCUSSION

Pesticides induce oxidative stress as well as alter the defense mechanisms of detoxification and the status of free radical scavenging enzymes.[21] These toxic compounds impair the cellular function, enzymes activity and produce cytotoxic changes through generation of ROS.[22] These free radicals also damage the cell components including proteins, lipids and DNA. In fact, the antioxidant enzymes e.g. SOD, CAT and GPx act simultaneously with non-enzymatic antioxidant GSHb (Tomlin, 1994).[23] They protect against the adverse effects of oxidative stress. SOD catalytically dismutates radicals to hydrogen peroxide and O₂. The findings showed that zinc and α- lipoic acid treatment markedly enhanced SOD level. These results strongly suggested that cypermethrin has the capability to induce free radicals and oxidative damage as evidenced by perturbations in various antioxidant enzymes.[24]

CAT and GPx are responsible for the catalytic decomposition of hydrogen peroxide to molecular oxygen and water (Tomlin, 1994).[23] GSH participates in the elimination of ROS, acting both as non enzymatic oxygen radical scavenger and as a substrate for various enzymes such as glutathione peroxidase.[25] In the present
study, cypermethrin treatment induced significant decrease in the activity of SOD and GPx in ovarian homogenate compared to control group. Also, significant changes in GSH and MDA in ovarian tissue were observed after cypermethrin-treatment compared to control rats. The change in SOD, GPx, GSH and MDA might be in response to increased oxidative stress and lipid peroxidation. According to Halliwell and Gutteridge[26] when a condition of oxidative stress strongly establishes, the defense capacities against ROS become insufficient. In turn, ROS also affects the antioxidant defense mechanisms, diminishes the intracellular concentration of GSH, increases lipid peroxidation and alters the activity of antioxidant enzymes e.g., SOD, CAT, GPx and GST. The changes in these oxidative stress biomarkers have been reported to be an indicator of tissue’s ability to cope with oxidative stress.[21][22]

Our results revealed that co-administration of zinc and α-lipoic acid with cypermethrin-treated rats restored the level of GSH, MDA and the activity of SOD and GPx towards the control values. The observed trend of normalization of GSH, SOD and GPx following zinc and α-lipoic acid treatment could possibly due to ROS scavenging effect of zinc and α-lipoic acid.

CONCLUSION
Therefore, the possible mechanisms of protective activity of zinc and α-lipoic acid with cypermethrin-induced toxicity could arise from their free radical scavenging activity, preventing lipid peroxidation and improvement of the antioxidant / detoxification system in ovary. It can be concluded that cypermethrin induced oxidative damage in female rat ovary. Zinc and α-lipoic acid provide significant protection against cypermethrin-induced oxidative stress and damage.

Conflict of Interest
The authors declare that there is no conflict of interest associated with this study.

ACKNOWLEDGEMENTS
The authors are thankful to Vidyasagar University, Midnapore for providing instrumental facilities. This study was supported by UGC Major Project grant (F.41-150/2012(SR)).

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