ToxEHS

Lipid Peroxidative Damage, Alterations in Antioxidant Status and Morphological Changes in Rat Erythrocytes on Lambda-cyhalothrin Exposure and Its Attenuation by Taurine

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Abstract

Lambda-cyhalothrin, a third generation type II pyrethroid, is used predominantly in agriculture production and animal husbandry. A study was conducted to investigate lambda-cyhalothrin induced oxidative stress, morphological changes of erythrocytes and other hematological biomarkers in rat and its amelioration by taurine, 2-amino ethane-sulfonic acid, a β-amino acid. Rats were randomly divided into six groups and lambda-cyhalothrin was orally administered at two dose levels (10.83 and 15.17 mg/kg body wt), singly or combined with pretreated taurine (50 mg/kg body wt) for consecutive 14 days. Treatment of Lambda-cyhalothrin resulted in an increase in malondialdehyde, oxidized glutathione level and depletion of reduced glutathione level, superoxide dismutase, catalase, glutathione-s-transferase and glutathione peroxidase in erythrocyte compared to control. Scanning electron microscopic studies showed a marked alteration in the morphology of Lambda-cyhalothrin treated erythrocytes. Lambda-cyhalothrin exposure also showed a significant decrease in erythrocyte count, hemoglobin percentage, haematocrit and red cell indices, whereas a significant increase in white blood cells and lymphocyte count was observed. However, pretreatment with taurine significantly restored the above said parameters. These findings revealed that lambda-cyhalothrin exposure produced oxidative stress, morphological changes of erythrocytes and other hematological biomarkers and its amelioration was accomplished by taurine through its reactive oxygen species scavenging activity.

Keywords: Lambda-cyhalothrin, Taurine, Oxidative stress, Antioxidant enzymes, Erythrocyte morphology, Haematological biomarkers

Introduction

Pesticides are used widely in agricultural production, animal husbandry, post-harvest technology as well as in public and animal health programmes. Indiscriminate use of pesticides results in a risk for the health and integrity of ecosystems enhances environmental pollution and causes health hazards to agricultural and industrial workers[1]. Because they are either recalcitrant or biodegraded very slowly in the environment, small residues of these pollutants have been found in drinking water, foods, even in human blood samples[2]. Pyrethroids are the synthetic analogues of natural pyrethrins derived from the ornamental plant *Chrysanthemum cinerariaefolium*[3,4]. These are lipophilic in nature that serves them to absorb through gastrointestinal and respiratory tracts and also make easier to be distributed into the lipid rich internal tissues like body fat, skin, liver, kidney, ovary and central and peripheral nervous systems[5].

Haematological studies play an important role in toxicological research[6]. These are used to determine systemic relationship and physiological adaptations of animals and commonly measured variables. Many reports are available regarding the pyrethroid-induced biochemical and physiological changes in target organs but very little attention has been paid to the effects of pesticides on non-target organisms.

Oxidative stress is a condition occurred by the disruption of oxidant-antioxidant balance which may cause deleterious cellular damages[7]. Antioxidants have defensive role against free radicals and oxidative attack. By converting oxidants into non-toxic molecules

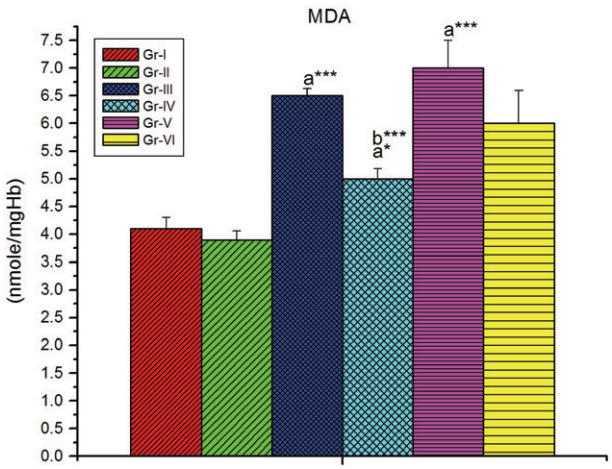

Figure 1. Effect of lambda cyhalothrin and taurine on MDA in control and experimental group of rat erythrocytes. Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IV and superscript c Gr-V versus Gr-VI (*indicates $p<0.05$, ***indicates $p<0.001$).

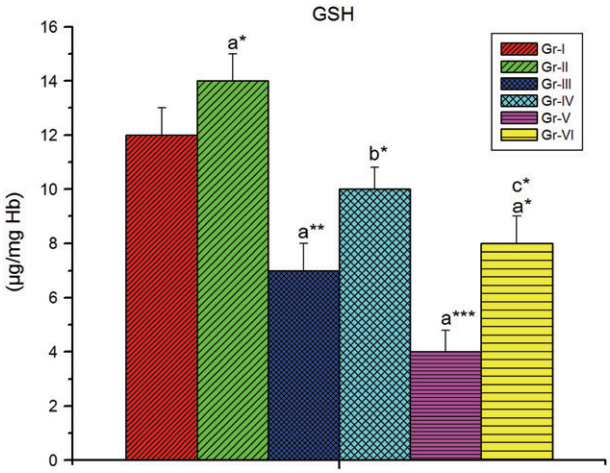

Figure 2. Shows the effect of lambda cyhalothrin and taurine on GSH in control and experimental group of rat erythrocytes. Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IV and superscript c Gr-V versus Gr-VI (*indicates $p<0.05$, **indicates $p<0.01$, ***indicates $p<0.001$).

it protects the organism from oxidative stress[8]. It has been already established that erythrocytes and their membranes are very much influenced by oxidative damage due to the presence of unsaturated fatty acids those are continuously exposed to high concentration of oxygen[9]. In spite of their well-equipped antioxidant defense system, erythrocytes may be injured, due to exposure to toxic chemicals like pyrethroids.

Lambda-cyhalothrin (LCT) appears to be a third generation type II pyrethroid, available in a number of commercial formulations[10]. It is used predominantly on cattle and sheep and to a smallerdegree in pigs and goats for the management of a broad range of ectoparasites. LCT shows significant toxicological changes in rabbit peripheral blood lymphocytes[11] but there is a lack of information regarding the LCT-induced morphological changes in mammalian erythrocytes.

Taurine, 2-aminoethanesulfonic acid is a major intracellular free β-amino acid, which is normally present in most mammalian tissues. It has the ability to maintain membrane stability, calcium homeostasis, osmoregulation and cellular detoxification[12]. Taurine reported to find clinical application against a variety of pollutants where cellular damage is a result of reactive oxygen species[13].

From our study, we have come to know that LCT is predominantly used in the agricultural production in different districts of West Bengal, India. There is a paucity of information on the toxic manifestations of lambda-cyhalothrin on oxidative stress and morphological changes of erythrocytes and the role of taurine in such conditions. Our interest in the present study to investigate lambda-cyhalothrin induced damage inflicted on the rat blood cells especially on the oxidative injury in erythrocytes, morphological changes of erythrocytes and other hematological biomarkers and to find out the possible ameliorative role of taurine.

Results

Alterations in Oxidative Stress Parameters

The results of the present study showed that malondialdehyde (MDA) level was significantly increased ($p<0.001$) in erythrocytes of the LCT treated groups compared to the control where MDA level decreased significantly ($p<0.001$) in combined (taurine + LCT-treated) groups (Figure 1).

The GSH levels in erythrocytes of high dose LCT-treated rat were altered significantly ($p<0.001$) compared to control (Figure 2). Co-administration of taurine to the low and high dose LCT-treated animals resulted in restoration of erythrocyte GSH levels significantly ($p<0.05$).

A significant increase ($p<0.001$) in GSSG level in high dose LCT treated rat erythrocytes was seen in Figure 3, which was significantly ($p<0.001$) reduced after pretreatment of taurine.

In Figure 4 the lambda cyhalothrin caused a significant decrease ($p<0.01$) in superoxide dismutase (SOD) activity in treated high dose group compared to the control. Pretreatment of taurine along with lambda cyhalo-

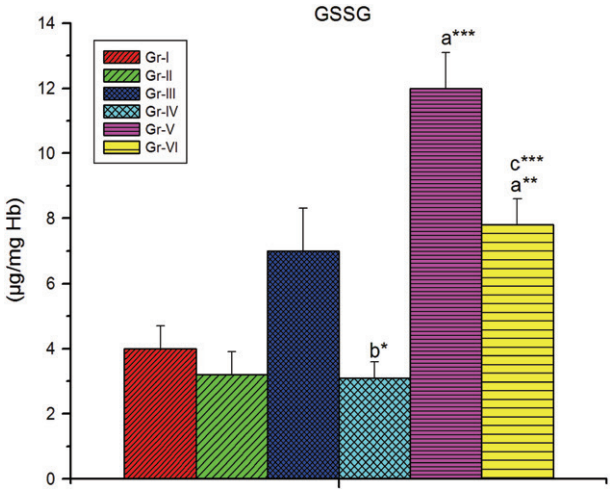

Figure 3. Effect of lambda cyhalothrin and taurine on GSSG in control and experimental group of rat erythrocytes. Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IV and superscript c Gr-V versus Gr-VI (*indicates $p < 0.05$, **indicates $p < 0.01$, ***indicates $p < 0.001$).

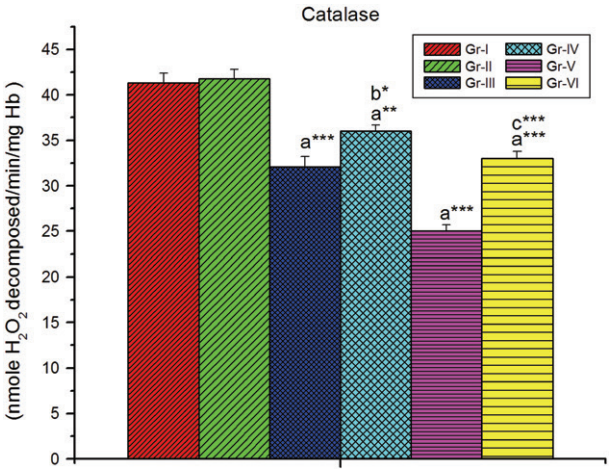

Figure 5. Effect of lambda cyhalothrin and taurine on catalase in control and experimental group of rat erythrocytes. Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IV and superscript c Gr-V versus Gr-VI (*indicates $p < 0.05$, **indicates $p < 0.01$, ***indicates $p < 0.001$).

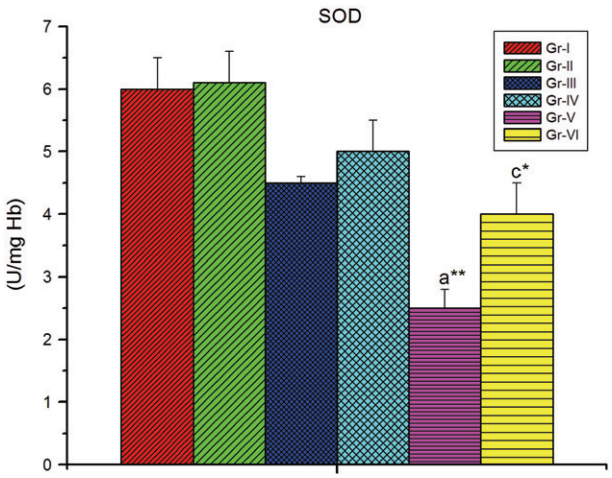

Figure 4. Shows the effect of lambda cyhalothrin and taurine on SOD in control and experimental group of rat erythrocytes. Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; and superscript c Gr-V versus Gr-VI (*indicates $p < 0.05$, **indicates $p < 0.01$).

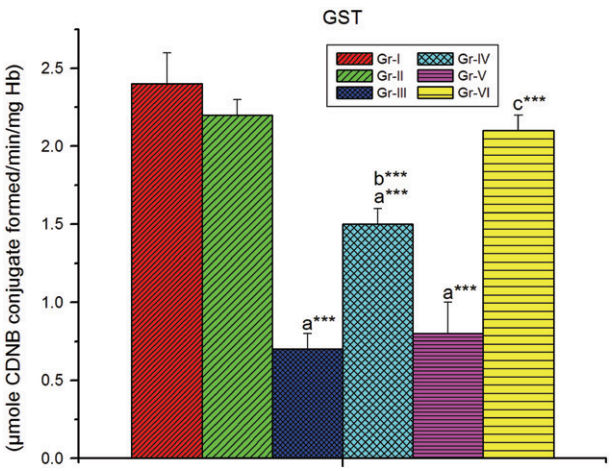

Figure 6. Shows the effect of lambda cyhalothrin and taurine on GST in control and experimental group of rat erythrocytes. Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IV and superscript c Gr-V versus Gr-VI (***indicates $p < 0.001$).

thrin improved the parameters significantly ($p < 0.05$).

Catalase (CAT) activity were diminished significantly ($p < 0.001$) in LCT treated group (Figure 5). Taurine supplementation along with lambda cyhalothrin caused increase CAT activity significantly ($p < 0.001$).

Significantly decreased ($p < 0.001$) of glutathione-s-transferase (GST) and glutathione peroxidase (GPx) activity were also observed in the LCT-intoxicated rats (Figures 6, 7). Pretreatment of taurine was found to exhibit a protective effect on the GST and GPx activity.

Scanning Electron Microscopic Observations of Rat Erythrocyte Morphology

Effects of LCT intoxication along with pretreatment

of taurine on rat erythrocytes were observed under scanning electron microscope and the results are depicted in Figure 8.

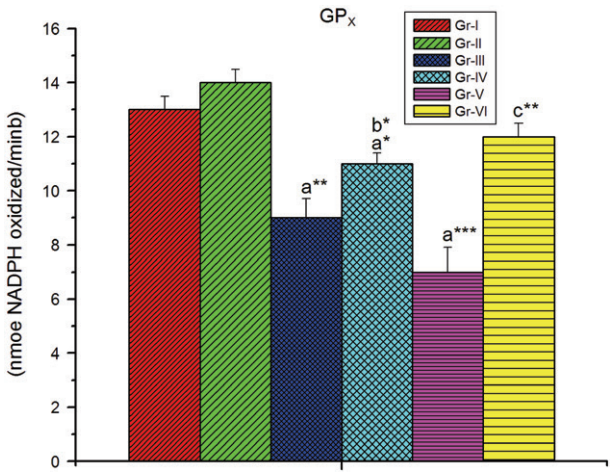

Figure 7. Effect of lambda cyhalothrin and taurine on in GPx control and experimental group of rat erythrocytes. Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IVand superscript c Gr-V versus Gr-VI (*indicates $p < 0.05$, **indicates $p < 0.01$ and ***represents $p < 0.001$).

Normal control animals showed perfect discocytes (Figure 8, Gr-I). No significant morphological abnormalities in blood cells were observed in the animals treated with taurine alone (Figure 8, Gr-II). However, animals treated with LCT in low doses showed morphological alterations from discocytes (D) to stomatocytes (S) and leptocytes (Figure 8, Gr-III). The red cells with stoma in middle part of the cells called stomatocytes along with folded bowl shaped. Few ovalocytes (O) were observed in high dose LCT treated animals (Figure 8, Gr-V). Certain irregularly created and contracted cells with numerous projections known as echinocytes (E) were also visible in the LCT treated high dose group animals (Figure 8, Gr-V). Dacrocytes (T) i.e. tear drop like structure were also found in this LCT treated high dose group (Figure 8, Gr-V). Protective effects of taurine were evident after the pretreatment of taurine followed by LCT, where the drastic alterations in the shape of the blood cells were restored close to the normal appearance of the cells (Figure 8, Gr-IV and Gr-VI). Despite all these protective effects of taurine, very low population of echinocytes were still present (Figure 8, Gr-I and Gr-II).

Effects on Haemogram

In LCT treated groups total erythrocyte count and haemoglobin percentage were decreased significantly

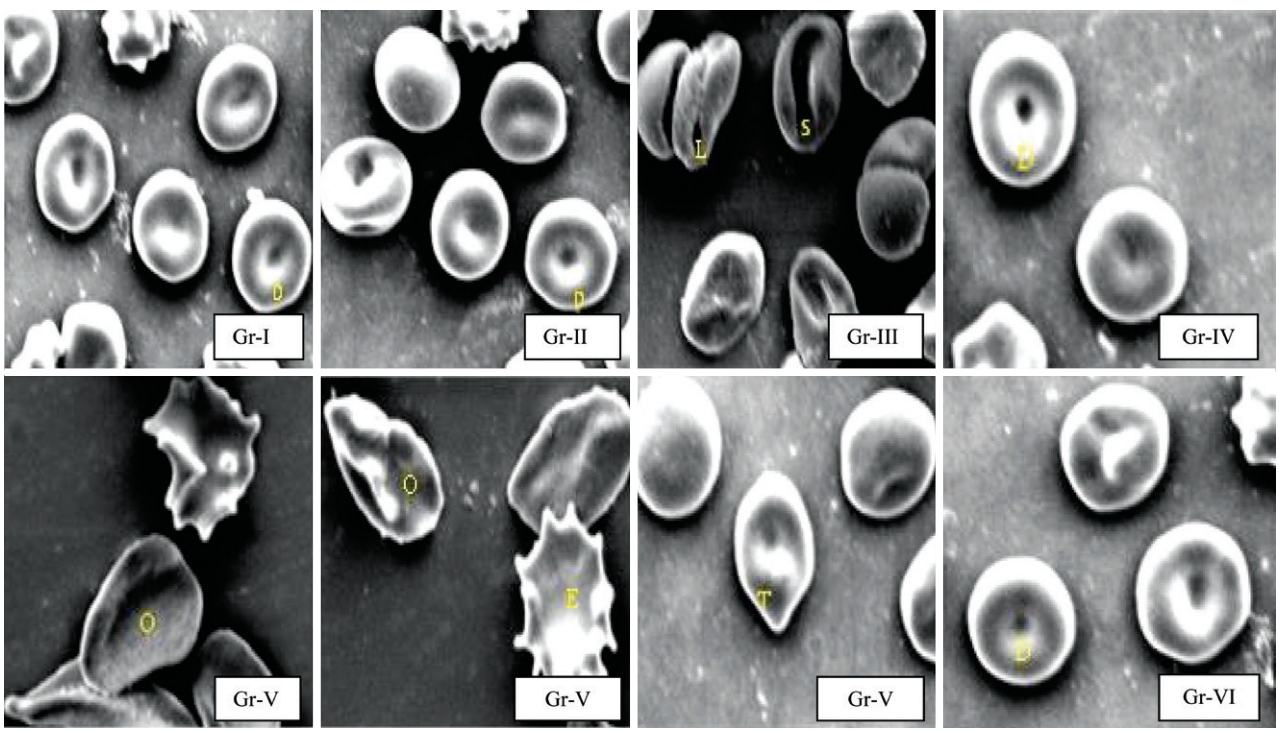

Figure 8. Scanning electron photomicrograph of rat erythrocytes. Discocytes (D), leptocyte (L), stomatocyte (S), ovalocyte (O), echinocyte (E), dacrocyte or tear drop like structure (T). Bar scale = 10 μm.

Table 1. Effect of lambda cyhalothrin and taurine on Total erythrocyte (RBC) count, Haemoglobin percentage, PCV (Packed cell volume), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) in control and experimental group of rat.

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
Total erythrocyte count ($\times 10^6$/mm^3)	7.3 ± 0.09	7.5 ± 0.15	6.3 ± 0.12a***	7 ± 0.1a*b**	5.8 ± 0.18a***	6.05 ± 0.14a***
Hb percentage (gm/dL)	14.73 ± 0.03	14.7 ± 0.05	11.86 ± 0.04a***	13.4 ± 0.07a***b***	10.31 ± 0.04a***	11.5 ± 0.15a***c***
PCV (%)	44 ± 0.14	44.8 ± 0.15a**	43.68 ± 0.07	44.48 ± 0.22a***b**	41.05 ± 0.31a***	42 ± 0.17a***c*
MCV (fl)	60.27 ± 0.26	59.73 ± 0.2	67.62 ± 0.08a***	61.43 ± 0.21a**b***	70.69 ± 0.02a***	68.86 ± 0.23a***c***
MCH (pg)	20.18 ± 0.02	19.6 ± 0.09a***	18.82 ± 0.03a***	19.14 ± 0.05a***b***	17.78 ± 0.06a***	18.85 ± 0.03a***c***
MCHC (%)	33.48 ± 0.05a***	32.81 ± 0.58a***	27.8 ± 0.58a***	31.16 ± 0.04a***b***	25.14 ± 0.57a***	27.37 ± 0.79a***c***

Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IV and superscript c Gr-V versus Gr-VI (*indicates $p < 0.05$, **indicates $p < 0.01$ and ***represents $p < 0.001$).

($p < 0.001$) (Table 1) which demonstrated the toxic effects of LCT. Pretreatment of taurine reduced the toxic effects of LCT and caused significant increase in total erythrocyte countand haemoglobin percentage. Red blood indices i.e. packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were decreased significantly ($p < 0.001$) in LCT treated group compared to control group (Table 1) whereas mean corpuscular volume (MCV) value was increased significantly in case of LCT-intoxicated group (Table 1). Taurine restored these parameters towards more or less control level (Table 1).

Effects on Leukogram

Leukocyte count and lymphocyte percentage (Table 2) were increased significantly ($p < 0.001$) in LCT treated groups. The results showed that the treatment of taurine to the rats exhibited significant decrease in leukocyte count and lymphocyte percentage compared to rats receiving only LCT. Neutrophil count also decreased significantly at dose-dependent manner in case of LCT treated rats. In LCT treated low and high dose groups, eosinophil and monocyte percentage were decreased significantly compared to that of control.

Discussion

In the present study lambda-cyhalothrin intoxication caused alterations in oxidative stress parameters in rat erythrocytes. Lambda-cyhalothrin also produced a significant variation in the morphological appearance of the rat erythrocytes and substantial changes in the haematological parameters. Protective effects of taurine were also reflected in this study.

The erythrocytes in normal physiological conditions are resistant to oxidative damage because of their well-equipped biological and protective mechanisms by enriched antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (GPx) and glutathione reductase (GR)[14,15]. However, under oxidative stress, the erythrocytes may be susceptible to oxidative damage due to the presence of heme-iron, PUFA and oxygen, which may initiate the reactions that induce oxidative changes in red blood cells[16]. Pyrethroids have been reported to induce oxidative stress, as shown by elevation of lipid peroxidation products[17,18]. LCT is an α-cyano moiety containing type-II pyrethroid which induces the oxidative stress. Nasuti and Prasanthi observed that oxidative damage was induced in erythrocytes due to lipophilicity of pyrethroids[19,20]. Increased malondialdehyde (MDA) level in erythrocytes treated with LCT is an agreement

Table 2. Effect of lambda cyhalothrin and taurine on total leukocyte count, lymphocyte count, neutrophil count, eosinophil count, and monocyte count in control and experimental group of rat.

Parameters	Group-I	Group-II	Group-III	Group-IV	Group-V	Group-VI
Total leukocyte count ($\times 10^6/\mu L$)	6.6 ± 0.12	6.6 ± 0.06	8.4 ± 0.05a***	7.9 ± 0.02a***b***	9 ± 0.23a***	8.3 ± 0.1a***c**
Lymphocyte count (%)	56.13 ± 0.13	56.05 ± 0.13	60.15 ± 0.26a***	58.0 ± 0.16a***b***	65.83 ± 0.17a***	62.05 ± 0.26a***c***
Neutrophil count (%)	36.9 ± 0.57	37.15 ± 0.83	34.0 ± 0.54a*	35.7 ± 0.28	29.97 ± 0.5a***	32.05 ± 0.5a***
Eosinophil count (%)	2.5 ± 0.06	2.6 ± 0.09	2 ± 0.1a**	2.4 ± 0.12b*	1.4 ± 0.06a***	2 ± 0.10a**c***
Monocyte count (%)	4.4 ± 0.19	4.2 ± 0.14	3.45 ± 0.16a**	3.9 ± 0.12a*b*	2.8 ± 0.10a***	3.0 ± 0.22a***

Results are expressed as Mean ± SEM. Analysis is done by one way ANOVA followed by multiple comparison two-tail t-tests. Superscript a Gr-I versus all other groups; superscript b Gr-III versus Gr-IV and superscript c Gr-V versus Gr-VI (*indicates $p < 0.05$, **indicates $p < 0.01$ and ***represents $p < 0.001$).

with the findings of Nasuti and Prasanthi[20]. The pretreatment of taurine in conjunction with lambda-cyhalothrin decreased the elevated level of MDA towards its normal limit. The normalization of erythrocyte MDA level following taurine pretreatment is very likely due to its antioxidant properties, as has been shown previously[21]. Reduced glutathione (GSH) is considered as an important biomolecule that acts against chemically induced oxidative stress. It eliminates reactive intermediates by reducing hydroperoxides in the presence of glutathione peroxidase. It also functions as a free radical scavenger and neutralizes the radicals which are involved in biological damage[22,23]. Therefore, the measurement of its activity is very important to assess the oxidative stress and antioxidant status induced by LCT. In the present study, GSH content decreased significantly in the erythrocyte of LCT treated rat compared to the control. Significant deterioration in the GSH level by LCT exposure is either due to increased utilization or decreased production of GSH. Taurine supplementation may cause an enhancement in GSH levels by directing cysteine into the GSH synthesis because cysteine is the precursor of GSH[24]. Increased GSSG level in case of LCT-treated erythrocytes and decreased oxidized glutathione (GSSG) in taurine treated erythrocyte also justify the previous findings. In this study, the decrease in the activity of superoxide dismutase, a copper-zinc-containing enzyme and CAT, a haem-containing enzyme in lambda-cyhalothrin intoxicated erythrocytes can be explained by the elevation of lipid peroxidation followed by increase in MDA content. LCT induced stress may be responsible for over production of ROS that increase singlet oxygen and peroxyl radicals, which diminish SOD and CAT by their huge utilization. Glutathione peroxidase (GPx), a selenium containing tetrameric glycoprotein found in mammalian erythrocytes helps to prevent lipid peroxidation of the cell membrane[25]. Lambda-cyhalothrin exposed erythrocytes showed a decrease in GPx, may be due to the depleted GSH, as the activity of GPx depends upon the level of GSH. Increased utilization of GPx to detoxify the pesticide induced free radicals, also support the above result[26]. Glutathione-s-transferase (GST) are detoxifying enzymes that catalyze the conjugation of a variety of electrophilic substrates to the thiol group of reduced glutathione, releasing less toxic forms[26]. The significant decrease of GST activity in erythrocytes after exposure of lambda-cyhalothrin may indicate enough detoxification of pesticide in rat erythrocytes. Taurine administration reversed all the abnormalities due to its stimulatory effect on endogenous antioxidants[27,28].

In this study we found the drastic alterations in the red cell morphology of the animals exposed to LCT.

The normal appearance of the erythrocytes was changed into many different forms including echinocytes, dacrocytes, stomatocytes and few spherocytes also. Echinocyte form is considered as a structurally pathological membrane defect, occurring also during the smear preparation. LCT may disrupt the structure of lipids located in the erythrocyte membrane[29]. This would mean that erythrocytes in individuals exposed to LCT shall not live their entire life span of 120-130 days, but are expected to be eliminated as echinocytes. This would lead to low hemoglobin levels due to LCT toxicity[30]. Stomatocyte has the stoma in middle part generally found in liver disease, also observed in LCT treated animals. However, due to lack of related studies in this field, the acceptable explanations for such findings are the abnormal erythropoiesis, or the defects on the erythrocyte membrane lipid bilayer. It may be due to insufficient haemoglobin formation, reduced water permeability across erythrocyte membranes, enhanced erythrocyte aging, the rate of oxygen release by erythrocytes, decreased thermo stability of erythrocyte, or augmented erythropoiesis to compensate anemia[31]. Pre-treatment of taurine followed by LCT improved the morphology of the red blood cells. The protective effects of taurine are most likely due to its function as a direct antioxidant by scavenging reactive oxygen radicals, inhibition of lipid peroxidation and as an indirect antioxidant by preventing changes in erythrocyte membrane permeability[32,33].

A significant change in haemogram, red cell indices and leukogram were observed in rats exposure to lambda-cyhalothrin indicates the physiological dysfunction of the haemopoietic system of rats. The decrease in erythrocyte counts observed with LCT treatment may be due to haemolysis as a result of type-II pyrethroid exposure which causes hemorrhages and reduced erythropoiesis[34]. Substantial decrease in erythrocyte counts and haemoglobin percentage could possibly be due to suppression of erythropoiesis and haem synthesis, and also due to destruction of erythrocyte in haemopoietic tissue[35,36]. Erythrocyte lysis is produced by chemicals that damage erythrocyte membrane, leading to oxidative injury to haemoglobin or may destroy the antioxidative protective mechanism. Increased haemolysis usually lead to reduction in haemoglobin, erythrocyte count are escorted by high reticulocytes counts, enlarged anisocytosis, increased red cell distribution width and volumes. Few authors have reported similar results with the treatment of cypermethrin in rats[35]. Haemoglobin percentage and haematocrit values have direct correlation to erythrocyte count[37] because of the synergistic link among these blood parameters in all vertebrates. Reduction in haemoglobin percentage in the present study could be due to the reduced biosynthesis of haem in bone marrow increased rate of destruction of erythrocyte or decreased rate of erythrocyte formation.

The packed cell volume (PCV) value indicates oxygen carrying capacity of blood and the degree of stress on animal health[38]. In high dose lambda-cyhalothrin treated group PCV level were decreased. In agreement with the present result decreased erythrocyte count, haemoglobin percentage and PCV levels were also reported in rats treated with deltamethrin[39].

In our study mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were decreased in lambda-cyhalothrin treated groups, which may be an indicator of macrocytic and hypochromic anemia[40]. Due to lower erythrocyte count, haemoglobin percentage and PCV in LCT treated animals; MCH and MCHC value were diminished. In the present study, the increase in mean corpuscular volume (MCV) and decrease in MCHC also indicates the possibility of macrocytic and hypochromic anemia[40,41], probably due to the increased activity of bone marrow and deficiency of some haemopoietic factors. Increased MCV may also be observed in regenerative anemia due to haemolysis and haemorrhages.

The increase in leukocytes was also noted in lambda-cyhalothrin intoxicated groups, may be due to the activation of immune system of the body[42]. This may have resulted in an increase in release of leukocytes from bone marrow storage pool into the blood. The primary function of leukocytes is to defend against foreign bodies, which is attained by leukocytosis and antibody production. Pathological leukocytosis may be formed due to exposure of chemicals or acute haemorrhages and haemolysis. Leukocytosis may be raised due to resistance of the animal for localization of the inflammatory response. Another possible cause of leukocytosis may be the severe haemorrhages in liver and lungs[41]. This increase may be related to an increase in lymphocyte percentage.

However pretreatment with taurine has a potent protective effect against lambda-cyhalothrin induced toxicity in haematological parameters of rats. The mechanism underlying haemato-protection of taurine may be related to its anti-anemic properties which strongly supports to haemopoiesis. A number of investigators reported that taurine protects several organs in the body against toxicity and oxidative stress due to exposure of heavy metals and other toxins as well as drugs[43,44].

Conclusions

In conclusion, it may be said that the lambda-cyhalothrin treatment creates toxicity by producing oxida-

tive stress and structural changes in erythrocytes and by alteration of haematological parameters in rat. Collectively, these data suggest that taurine pretreatment can potentially be considered as an intervention in human subject against haematological dysfunction with accidental exposure of lambda-cyhalothrin and related pyrethroids.

Materials and Methods

Chemicals

Lambda-cyhalothrin (LCT) 5% emulsifiable concentrate (EC) was procured from RPC Agro Industries, Kolkata. Taurine was purchased from Sigma Aldrich Inc., USA. All other chemicals used were of analytical grade and were purchased from Merck India Ltd, Himedia India Ltd.

Animal Care

For the present study, mature Wistar male albino rats (weighing 130-150 g) were taken and the animals were kept under controlled temperature (25 ± 2°C) and light conditions (12h-light-dark cycle with free access to water and standard laboratory feed. The rats were acclimatized for a week before beginning the experiments. All the animal experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC), registered under Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India. All animal treatment and surgical procedures were carried out in accordance with the relevant laws and guidelines of the CPCSEA.

Treatment Protocol

The animals were divided into six groups and each group contains six rats.

Group	Dose
Gr-I: Distilled water control	No treatment
Gr II: Taurine control	50 mg/kg body wt.
Gr III: LCT low dose	1/7 of LD_{50} value i.e., 10.83 mg/kg body wt.
Gr IV: Taurine + LCT low dose	50 mg/kg body wt. + 10.83 mg/kg body wt.
Gr V: LCT high dose	1/5 of LD_{50} value i.e., 15.17 mg/kg body wt.
Gr VI: Taurine + LCT high dose	50 mg/kg body wt. + 15.17 mg/kg body wt.

Oral LD_{50} dose of lambda-cyhalothrin for male rats (75.85 mg/kg body weight) was obtained from the report of Sharma *et al*. (2010)[45] and it was again verified in laboratory. Final doses and the duration of the study for the present treatments were selected by our preliminary investigations. The selected doses produced the toxicity in animals but lethality was not seen. Taurine was applied in our experiments at the dose level of 50 mg/kg body wt. This dose was effectively used to ameliorate the toxicity induced by various xenobiotics[46,47]. Taurine was pre-treated before 1 hr of LCT exposure. Lambda-cyhalothrin, taurine and distilled water were administered once daily by oral gavage for 14 consecutive days. Animal's weight was taken daily and the dose was adjusted accordingly.

Sample Collection

After the last day of treatment, all the animals were sacrificed under light anaesthesia and blood samples were collected from all the treated and control animals for the assessment of oxidative stress parameters, erythrocyte morphology and other haematological parameters.

Separation of Erythrocytes

Using ethylene-diamine-tetra-acetic acid (EDTA) as an anticoagulant, 2 mL of blood was collected from the hepatic vein of each rat and was poured upon the same amount of histopacque-1077. Then it was centrifuged at 2000 rpm for 30 minutes to separate the erythrocytes. By removing plasma and leukocytes only erythrocytes were suspended in phosphate buffer (0.1 M, pH 7.4) at 5% (v/v) concentration and were used for the assay of oxidative stress in rat erythrocytes.

Study on Oxidative Stress Parameters in Rat Erythrocytes

Estimation of Malondialdehyde (MDA)

MDA of erythrocyte suspension was determined according to modified method of Ohkawa *et al*.[48]. The reaction mixture contained 1 mL of erythrocyte suspension with 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of acetate buffer (20% pH 3.5), and 1.5 mL of aqueous solution of thiobarbituric acid (0.8%). Immediately the mixtures were boiled for 60 min at 95°C. After heating the red pigment was produced, that was extracted with 5 mL of n-butanol-pyridine (15 : 1) and centrifuged at 5000 rpm for 10 min at room temperature. The absorbance of supernatants was taken at 535 nm.

Estimation of Reduced Glutathione (GSH)

Reduced glutathione was carried out according to the method of Griffith, 1981[49]. Briefly, 100 µL of sulfosalicylic acid was mixed with 200 µL of erythrocyte

suspensions and the mixture was centrifuged for 10 min at 3000 rpm. Then 1.8 mL of DTNB was added with the supernatant and shaken well. Reading of the supernatants was taken at 412 nm. The glutathione level in erythrocytes was expressed as μg/mg Hb.

Estimation of Oxidized Glutathione (GSSG)

Oxidized glutathione was determined according to the method of Griffith, 1980[50]. At first 100 μL of erythrocytes suspensions was mixed with 2 μL of 2-vinyl pyridine and was incubated for 1 hr at 37°C. Then 250 μL of sulfosalicylic acid was added to it and was kept in room temperature for 30 min. It was then centrifuged at 2000 rpm for 10 min. After that 2 mL of DTNB was mixed with 200 μL of supernatant and the absorbance was taken at 412 nm within 1 min.

Superoxide Dismutase (SOD) Activity

SOD activity of erythrocyte was measured according to the method of Marklund and Marklund[51]. Briefly, in a spectrophotometric cuvette, 2 mL of Tris-HCl, 20 μL of 10 mM pyrogallolin the presence of EDTA and 20 μL of erythrocyte suspensions were added and the reading was taken at 420 nm for 3 min. The enzyme activity was estimated by measuring the percentage inhibition of the pyrogallol autoxidation by SOD.

Assay of Catalase (CAT) Activity

Catalase activity was measured by adding 0.5 mL of H_2O_2, 2.5 mL of double distilled water and 40 μL of erythrocyte sample (in 0.05 M Tris-HCl) in a glass cuvette. After mixing, six readings were noted at 240 nm at 30 sec interval[52].

Evaluation of Glutathione-s-transferase (GST) Activity

Glutathione-s-transferase (GST) activity in erythrocytes was estimated spectrophotometrically according to the method of Habig *et al*.[53]. Reaction mixture contained 0.1 mL of erythrocytes suspensions, 2.8 mL of PBS, 0.1 mL of GSH and 50 μL of 60 mM CDNB. All the contents were taken in a cuvette and reading was noted at 34 nm. The values were expressed in μmol CDNB conjugate formed/min/per milligram of Hb for erythrocytes.

Assay of Glutathione Peroxidase (GPx) Activity

Determination of GPx was carried out by the method of Rotruck *et al*.[54]. Briefly, the reaction mixture contained 0.2 mL of 0.4 M Phosphate buffer (pH-7), 0.1 mL of 10 mM sodium azide, 0.2 mL of tissue homogenized in Phosphate buffer (pH-7), 0.2 mL of 4 mM reduced glutathione, and 0.1 mL of 2.5 mM hydrogen peroxide (H_2O_2). The contents were incubated for 10 min at 37°C, and 0.4 mL of 10% TCA was added to stop the reaction and centrifuged at 3200 rpm for 20 min. Then 1 mL of 5,5′-dithiobisnitrobenzoic acid (DTNB) and 3 mL of disodium hydrogen phosphate (Na_2HPO_4) were added to supernatant and the optical density was measured at 420 nm.

Scanning Electron Microscopic (SEM) Study of Rat Erythrocytes

Scanning electron microscopic study[55] of rat erythrocytes were done by taking blood sample from each animal. Erythrocytes were separated using Histopacque-1077 and then 500 μL of erythrocytes were immediately fixed in 2.5% glutaraldehyde made in 0.1 M phosphate buffer (pH 7.4). After 1 h of fixation, cells were centrifuged at 1000-1500 rpm and pellets were suspended in triple distilled water. After single washing, the final pellet was again suspended in triple distilled water. A drop of the sample was smeared on the metallic SEM stubs, loaded with a conductive silver tape on its top. The stubs were then coated with gold to a thickness of 100 Å using a sputter-ion coater, for 4-5 min and the specimens were finally ready to observe under scanning electron microscope.

Total Erythrocyte Count (Red Blood Cell Count)

Total erythrocyte count[56] was done by diluting blood in 1 : 200 dilution with RBC dilution fluid and then total erythrocytes were counted in Neubaur haemocytometer chamber and were expressed as $\times 10^6\,mm^{-3}$.

Estimation of Haemoglobin Percentage

The haemoglobin percentage was measured by cyanmethemoglobin method[57]. Briefly, 20 μL of blood was transferred into a test tube and 5 mL of Drabkin's solution was added. The optical density was measured at 540 nm.

Packed Cell Volume (PCV)

Packed cell volume (PCV) was measured by taking anticoagulated whole blood, which was centrifuged at 3000 rpm for 30 minutes in a Wintrobe's tube. The erythrocytes were settled down at the bottom[56]. The PCV was determined by the height of erythrocyte column which is directly read from the graduation mark on the Wintrobe's tube.

Red Cell Indices

Red cell indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured from the total erythrocyte count, haemoglobin percentage and haematocrit value[56].

I. Mean corpuscular volume (MCV): The average volume of erythrocyte is known as mean corpuscular

volume and is expressed in 'Femtolitres'.

$$\text{MCV} = \frac{\text{Haematocrit (\%)}}{\text{RBC count in million}} \times 100$$

II. Mean corpuscular haemoglobin (MCH): The average weight of haemoglobin content in erythrocyte is called mean corpuscular haemoglobin and is expressed in 'Picograms'.

$$\text{MCH} = \frac{\text{Haemoglobin (g/dL)}}{\text{RBC count in million}} \times 10$$

III. Mean corpuscular haemoglobin concentration (MCHC): Expression of the average haemoglobin concentration per unit volume of packed red cell is defining as mean corpuscular haemoglobin concentration and is expressed in '%'.

$$\text{MCHC} = \frac{\text{MCH}}{\text{MCV}} \text{ or } \frac{\text{Hb (gm/dL)}}{\text{Hct (\%)}} \times 100$$

Total Leukocyte Count (TLC)

Total leukocyte count[56] was done by diluting blood in 1 : 20 dilution with white blood corpuscle (WBC) dilution fluid and then total leukocytes were counted in Neubaur haemocytometer chamber.

Differential Leukocyte Count (DLC)

Thin blood smear was made by anticoagulant-added whole blood in a clean glass slide and was stained with Leishman's stain and then was observed under oil immersion objective of the microscope. An area of the blood smears slightly before than 'tail end' was chosen where the morphology of the white cells is clearly visible. The percentage of granulocytes and agranulocytes were calculated[56].

Statistical Analysis

The data were analyzed to achieve mean values and standard errors for all treated and control samples. Statistical analyses of the collected data were completed by a one-way analysis of variance (ANOVA), followed multiple comparison two tail t-test for analysis between groups, using Origin 6.1 software. Results were presented as mean ± SEM. Difference was considered statistically significant when $p < 0.05$.

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Conflict of Interest

The authors declare no conflict of interest.

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