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Alteration in estrous cycle, lipid peroxidation and antioxidant status in female rat after exposure to lambda cyhalothrin and its attenuation by taurine

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Abstract: Lambda cyhalothrin, a broad spectrum pyrethroid, is being extensively used against a wide range of pests in agricultural practices. The present study was highlighted to investigate the effects of the lambda cyhalothrin on estrous cycle and oxidative stress in rat ovary, also to find out the protective role of taurine against lambda cyhalothrin induced ovarian toxicity. Mature 36 female Wistar rats were divided in six groups and they were received oral administration of lambda cyhalothrin at two dose levels (8.33 and 16.66 mg/kg body wt) for consecutive14 days. Taurine (50mg/kg body wt) was treated before lambda cyhalothrin administration in the combined taurine-lambda cyhalothrin treated groups. Lambda cyhalothrin caused significant alterations in estrous cycle with an increase in diestrous index compared to control rats. Lambda cyhalothrin treatment also produced oxidative stress in ovary by significant increase in malondialdehyde level, accompanied by a reduction in reduced glutathione and antioxidant enzymes (superoxide dismutase, catalase, glutathione-s-transferase, glutathione peroxidase, glutathione reductase). The present study revealed that the pretreatment of taurine was able to ameliorate lambda cyhalothrin induced ovarian oxidative stress.

Key Words: Lambda cyhalothrin; Taurine; Estrous cycle; Oxidative stress; Antioxidant enzymes.

INTRODUCTION

The undesired effects of pesticide have been recognized as a serious public health concern during the past decades. Insecticide exposure have known to cause problems and outbreak of diseases among animals and human[1].Chronic neurological syndromes, malignant tumors, immunosuppressive and teratogenic effects in experimental animals are often caused by prolonged exposure to insecticides[2].Ovary, which has a key role in female reproduction, is one of the affected organs in mammals [3,4].

Disease vectors such as cockroaches, mosquitoes, ticks and flies are controlled by pyrethroids, structural derivatives of naturally occurring pyrethrins and have greater potency over organochlorines, organophosphates and carbamates. They affect the nerve fiber of the disease vectors by binding to a protein that regulates the voltage-gated sodium channel.

During the past few years, quantification of free radical generation and antioxidant defense status has become an important aspect in assessing the toxicity of any chemical. One of the important molecular mechanisms involved in pesticide-induced toxicity assumed to be the lipid per-oxidation[5-12]. In normal physiological condition, reactive oxygen species (ROS) and antioxidants remain more or less in balanced level. When this balance is disturbed towards an increase in ROS, oxidative stress occurs. ROS affect multiple physiological processes from oocyte maturation to fertilization; produce alterations in metabolic reactions, embryo development and pregnancy.

Lambda cyhalothrin(LCT), a third generation type-II synthetic pyrethroid, is widely used to control insect pest in agriculture, public health, homes and gardens.Due to its rapid metabolism and excretion it may create problems in non-target species if applied indiscriminately. LCT has been found to accumulate in biological membranes leading to oxidative damage due to its lipophilic nature[13]. The toxicity of LCT to mammals and its ability to induce oxidative stress *in vivo* and *in vitro* have been established from various reports[14-18]. However, information on the status of lipid peroxidation and antioxidant defence system of this pesticide in ovary is publically unavailable.

Taurine, 2-aminoethanesulphonic acid is an essential amino acid and is present at high concentrations in many tissues. It plays important roles in various physiological functions including bile acids conjugation, calcium level modulation and maintenance of osmolarity, antioxidation and membrane stabilization [19, 20]. Its beneficial effects in various physiological and pathological conditions by reducing reactive oxygen species[21-23]and preventing DNA damage[24, 25] were also reported.

Female reproductive function specially the oxidative status of female reproductive system can be altered by the exposure of toxic chemicals like pyrethroids. The present study was carried out to assess the effect of oral exposure of LCT on estrous cycle as well as on ovarian oxidative status of female Wistar rat and to find out whether there is any the protective role of taurine against the LCT induced effects.

MATERIALS AND METHODS

Chemicals and Reagents:

Lambda cyhalothrin 5%Emulsifiable Concentrate (EC) was procured from RPC Agro Industries, Kolkata. Taurine and 1, 2dichloro-4-nitrobenzene (CDNB) was purchased from Sigma-Aldrich. Thiobarbituric acid, 5, 5' Dithiobis-2-nitrobenzoic acid (DTNB), ethylene di tetra acetic acid (EDTA), oxidized and reduced forms of glutathione and hydrogen peroxide were all purchased from Sigma Chemical, USA. All other chemicals used were of the finest analytical grade.

Animal care and treatment:

Mature 36 Wistar female albino rats weighing 130-150g were acclimatized for 1 week before the treatments at

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temperature of 25°±2°C with 12 hrs light-dark cycle. The animals were given the standard laboratory feed and water adequately throughout the period of experimentation. Rats were randomly divided into six groups, of six animals each. The experimental protocol was approved by the Institutional Animal Ethics Committee. The experiments were designed as:

1. Group I: Control (2 ml distilled water /kg body wt.)
2. Group II: Taurine control (50mg/kg body wt)
3. Group III: Lambda cyhalothrin low dose (8.33mg/body wt.)
4. Group IV: Taurine (50mg/kg body wt) +lambda cyhalothrin low dose (8.33mg/body wt.)
5. Group V: Lambda cyhalothrin high dose (16.66mg/body wt.)
6. Group VI: Taurine (50mg/kg body wt) + lambda cyhalothrin high dose (16.66mg/body wt.)

After one hour of the treatment of taurine (50mg/kg body wt), lambda cyhalothrin was administered at two dose levels (8.33mg, and 16.66mg /kg body wt.)[26] for consecutive 14 days. All animals were observed at least once daily to notice whether there was any behavioral change or signs of intoxications. Food and water consumption were monitored daily. Animal's weight was taken daily and the dose was adjusted accordingly.

Sample collection

Body weight of each rat was taken during the treatment period and before sacrifice. All rats were sacrificed by rapid decapitation after 24 hrs of the last dose. The ovaries were removed immediately and adhering fats were cleaned. Then weights of the ovaries were recorded and stored properly for the determination of oxidative stress biomarkers.

Estimation of Ovarian index

Ovarian index was measured by using the following formula:

$$\text{Ovarian index} = \frac{\text{Ovarian weight (g)}}{\text{Body weight (g)}} \times 100$$

Study of Estrous cycle[27]:

Vaginal smear histology is used to understand the estrus cycle as an index of ovarian functions. A detailed comparative estrous cycle study was designed in female rats exposed to two above mentioned doses of LCT and also pretreatment of taurine.

Collection of Vaginal smear:

The vaginal smear was collected daily in the morning (11.00-11.30a.m) through a Pasteur pipette filled with normal saline (0.9%NaCl) by inserting the tip into the rat's vagina. Vaginal lavage was taken on glass slides. A different glass slide was used for each rat for the specific dose. One drop was collected with a clean tip from each rat.

Staining and microscopic observations[28]:

The slides containing the vaginal smear were stained with hematoxylin and eosin. All the stained slides were examined under light microscope. The phases of estrous cycle were confirmed depending on the presence of different characteristic cells. The following formula was used for measuring the diestrous index:

$$\text{Diestrous index} = \frac{\text{No. of days with clear diestrous smear}}{\text{Total duration of treatment}} \times 100$$

Estimation of oxidative stress parameters:

Ovarian malondialdehyde (MDA)[29]: The reaction mixture consisted of 0.5ml of ovarian homogenate,1 ml of TBA(0.8%)-TCA (20%) mixture. Then the mixture was boiled for 45 min at 95°C. After cooling at room temperature it was centrifuged at 4000 rpm for 10 min and the reading was taken at 535nm.

Ovarian reduced glutathione (GSH)[30]: The assay mixture contained 200µl of ovarian homogenate and 100 µl of sulfosalicylic acid. The mixture was centrifuged for 10 min at 3000 rpm. Then 1.8 ml of DTNB was added with the supernatant and was shaken well. Reading was taken at 412-420nm.

Ovarian oxidized glutathione (GSSG)[31]: 100µl of ovarian homogenate was mixed with 2µl of 2-vinyl pyridine and was incubated for 1hr at 37°C. Then 250µl of sulfosalicylic acid (4gm %) was added with it and was kept in room temperature for 30min. It is centrifuged at 2000 rpm for 10 min. Then 200 µl of supernatant was added with 2 ml of DTNB (4mg %) and the reading was taken at 412 nm within 1min.

Ovarian superoxide dismutase (SOD)[32]: At first 2 ml of 50 mM Tris Hcl, 20µl of 10 mM pyrogallol and 20 µl of ovarian homogenates were poured in a spectrophotometric cuvette and the absorbance was noted in the spectrophotometer at 420 nm for 3 min. The enzyme activity was estimated by measuring the percentage inhibition of the pyrogallol autoxidation by SOD.

Ovarian catalase (CAT)[33]: The reaction mixture consisted of 0.5ml of H_2O_2, 2.5ml of double distilled water and 40µl of ovarian homogenate prepared in 0.05M trisHCl and was taken in a cuvette. After mixing, six readings were noted at 240nm in 30sec interval.

Ovarian glutathione-s-transferase (GST)[34]: Ovarian homogenate (0.1 ml), 2.8ml of PBS,0.1ml of GSH and 50µl of 60mM CDNB were taken in a cuvette and reading was noted at 340nm.

Ovarian glutathione peroxidase (GPx)[35]: Ovarian tissue homogenate was added with 2.5 mM H_2O_2, 0.4 M sodium phosphate buffer, 10 mM sodium azide and reduced glutathione and volume made upto 2 ml with distilled water and was incubated for 5min at 37°C. Then 10% TCA was mixed with reaction mixture. After that the mixture was centrifuged and the supernatant was mixed with 1 ml of DTNB and 3 ml of Na_2HPO_4. Reading was taken at 412nm.

Ovarian glutathione reductase (GR)[36] : 2ml of GSSG, 20µl of 12 mM $NADPHNa_4$ and 2.68ml of PBS were added with 100µl of ovarian homogenate and the reading was taken at 340nm.

RESULTS

Effects on ovarian index

The ovarian index of LCT exposed rats was decreased significantly ($p<0.001$) in a dose dependent manner compared to the rats of the control group (Table-1). Taurine increased the ovarian index of LCT induced rats significantly.

Effects on estrous cycle

Result showed a significant decrease ($p<0.001$) in frequency of estrous cycle. In case of treated animals, most of the rats exhibit abnormal estrous cycle. The duration of proestrous, estrous and metestrous of LCT treated rats of two dose groups were reduced significantly in a dose dependent manner compared to the rats of the control group (Table-2). In high dose LCT treated group, diestrous phase came earlier and persisted for a long time compared to the low dose LCT treated group. A significant increase was observed in the duration of diestrous phase and diestrous index of treated rats in a dose dependent manner (Table-2). Taurine decreased the duration of diestrous phase and diestrous index of LCT exposed rats significantly ($p<0.001$).

Effects on oxidative stress

The effect of taurine on ovarian malon-di-aldehyde (MDA) in lambda cyhalothrin exposed female albino rat is shown in Figure 1. In LCT treated group, MDA content increased significantly ($p<0.001$) compared to the control group in dose dependent manner where treatment of taurine decreased the LCT toxicity and restored the normal status of the ovary to a great extent.

Figure 2 shows the effect of taurine on ovarian GSH in lambda cyhalothrin exposed female albino rat. From this figure it is observed that GSH were decreased significantly ($p<0.001$) in LCT treated groups compared to the control which were alleviated by taurine.

The effect of taurine on ovarian GSSG level in lambda cyhalothrin induced female albino rat is shown in Figure 3. GSSG level was significantly higher ($p<0.001$) in rats of LCT treated high dose group. Taurine restored it to a good extent.

As presented in Figure 4, the activity of SOD in the LCT treated low and high dose were significantly decreased ($p<0.05$) and ($p<0.001$) compared to the control group. However, pretreatment with taurine resulted in a significant increase in the activity of SOD in low dose ($p<0.05$) and in high dose ($p<0.001$) group animals respectively.

As depicted in Figure 5, the activities of CAT in the LCT treated low and high dose groups were significantly ($p<0.001$) decreased compared to the control group. However, the activity of CAT was significantly increased by taurine pretreatment in low ($p<0.01$) and ($p<0.05$) high dose group animals.

Activity of GST on LCT treated low and high dose animals is shown in Figure 6. GST level was increased significantly at dose dependent manner in case of LCT treated group compared to control, where pretreatment of taurine restored the normal status.

As shown in figure 7, the activity of glutathione peroxidase and glutathione reductase in the LCT treated group was significantly decreased in ovary. However, pretreatment with taurine resulted in a significant increase in the activity of glutathione peroxidase and reductase.

Table 1: Effect of taurine on ovarian index in lambda cyhalothrin exposed female albino rat.

Group	Ovarian Index
Group-I (control)	0.030±0.0003
Group-II (Taurine control)	0.029±0.0004
Group-III (LCT, 8.33mg/body wt.)	0.027±0.0003a***
Group-IV (Taurine+LCT, 8.33mg /body wt.)	0.0286±0.00055a***b*
Group-V (LCT, 16.66mg/body wt.)	0.024±0.00031a***
Group-VI (Taurine+LCT, 16.66mg /body wt.)	0.028±0.001a***c***

Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, b, c) differ from each other significantly. 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 $p<0.001$).

Table 2: Effect of taurine on estrous cycle in lambda cyhalothrin exposed female albino rat.

Group	No of estrus cycle	Proestrous	Estrous	Metestrous	Diestrous	Diestrous Index (DI)
Group-I	5.7±0.2	5.3±0.2	7.5.2±0.3	4.5±0.3	12±0.4	84.02±5.4
Group-II	5.3±0.2	5.2±0.4	4.2±0.3a***	4.3±0.2	11.7±0.3	82.66±2.3
Group-III	2.5±0.4a***	2.8±0.4 a***	6±0.4a*b*	3.5±0.4	18±0.6a***	128.57±4.1a***
Group-IV	4±0.25a***b*	4.2±0.4a**b*	3.3±0.3a***	4±0.4	15.5±0.2a***b**	120.24±5.6a***
Group-V	1.3±0.2a***	1.33±0.3a***	3.3±0.3a***	1.2±0.2a***	23±0.4a***	164.28±5.8a***
Group-VI	2.8±0.16a***	2.7±0.3a***c*	4.8±0.4a***c*	3.3±0.2a*c***	18.16±0.5a***c***	135.7±3.7a***c**

Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, b, c) differ from each other significantly 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 $p<0.001$).

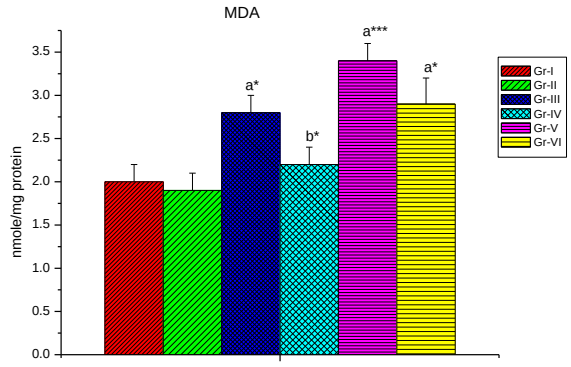

Figure 1: Effect of taurine on ovarian Malon-di-aldehyde (MDA) in lambda cyhalothrin exposed female albino rat.Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a,b) differ from each other significantly.Superscript a, Group-I versus all other groups; Superscript b Group-III versus Group-IV. Asterisks represents the different level of significance (* indicates p<0.05, *** indicates p<0.001).

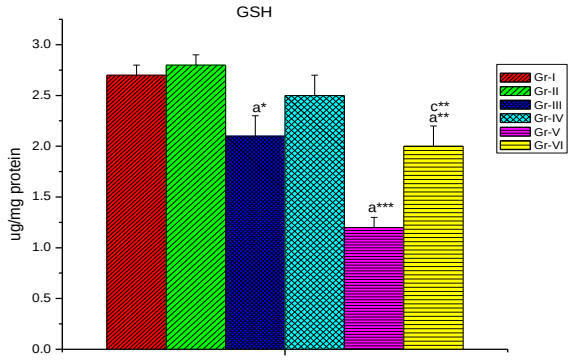

Figure 2: Effect of taurine on ovarian Reduced glutathione (GSH) in lambda cyhalothrin exposed female albino rat.Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, c) differ from each other significantly.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).

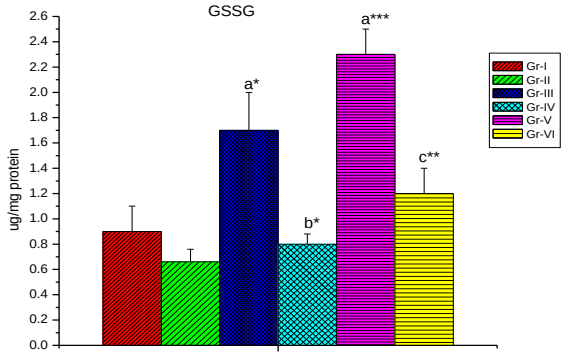

Figure 3: Effect of taurine on ovarian Oxidized glutathione (GSSG) in lambda cyhalothrin exposed female albino rat.Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, b, c) differ from each other significantly.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).

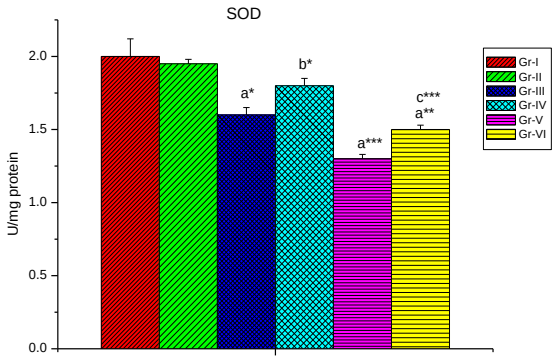

Figure 4: Effect of taurine on ovarian Super oxide dismutase (SOD) in lambda cyhalothrin exposed female albino rat. Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, b, c) differ from each other significantly. 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).

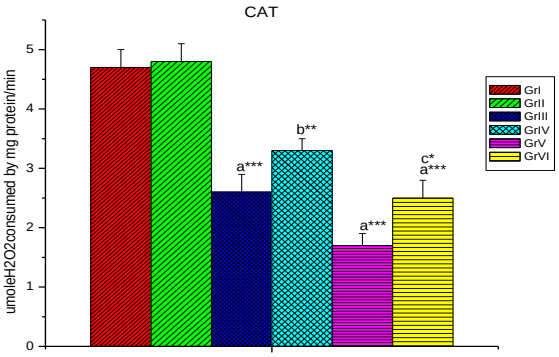

Figure 5: Effect of taurine on ovarian Catalase (CAT) in lambda cyhalothrin exposed female albino rat. Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, b, c) differ from each other significantly.Superscript 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).

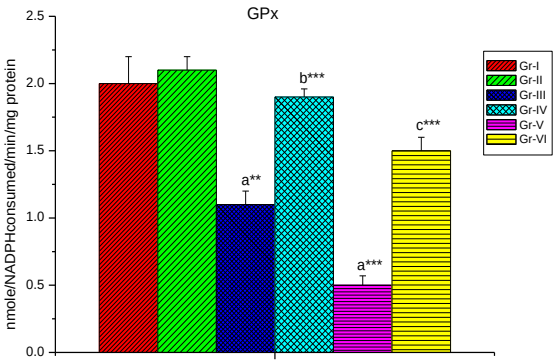

Figure 6: Effect of taurine on ovarian Glutathione peroxidase in lambda cyhalothrin exposed female albino rat. Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, b, c) differ from each other significantly 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).

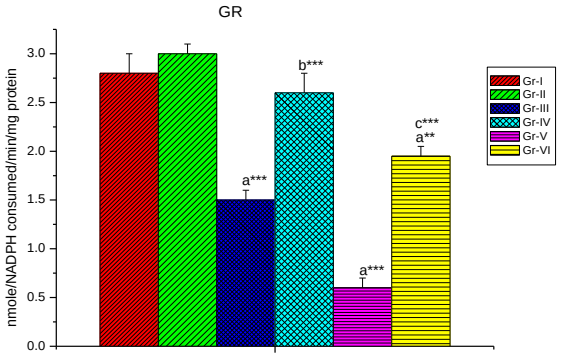

Figure 7: Effect of taurine on ovarian Glutathione reductase in lambda cyhalothrin exposed female albino rat. Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, b, c) differ from each other significantly 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).

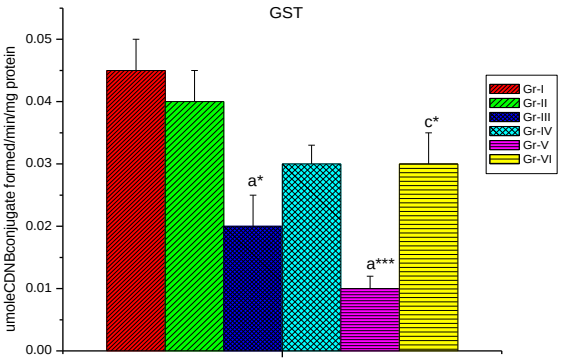

Figure 8: Effect of taurine on ovarian Glutathione-S-transferase (GST) in lambda cyhalothrin exposed female albino rat. Results are expressed as Mean±SEM (N=6). Analysis is done by ANOVA followed by multiple comparison two-tail t-tests. Probability values with different superscripts (a, c) differ from each other significantly.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<0.001).

DISCUSSION

The present study was planned to evaluate the adverse effects of LCT on female rats and its attenuation by taurine. In this study we have examined the ovarian index and estrous cycle as the basic benchmarks for the ovarian toxicity. In this study, a decrease in ovarian index in LCT treated rats may be due to decreased number of ovarian germ cells. Other possible reason for LCT induced loss of body wt. and ovarian weights expressed as ovarian index may be due to endocrine disruption, inhibition of steroidogenic enzyme activity. Inadequate female gonadotropins are also responsible for the reduction in body and ovarian weights by inducing the atrophy of somatic and ovarian tissues. In addition to this, one of the most common reasons of tissues damage is oxidative stress which was seen in LCT exposed rats in this study.

In our study we have observed that the rats in control group shows regular estrous cycle but reduction in the estrous cycle was noticed in the LCT exposed rats. The duration of proestrus, estrous and metestrous also decreased with a consequent increase in the duration of diestrous in each estrous cycle in a dose dependent manner compared to control rats. Moreover, the diestrous index has been increased significantly in LCT treated rats. All the above findings were in agreement with previous observations[37].

LCT induced toxic manifestations may also be associated with induction of oxidative stress through the formation of free radicals and alteration in antioxidant systems. It was reported that LCT significantly increased the level of MDA in the liver and kidneys of rats, whereas the activity of antioxidant enzymes (SOD, CAT) was decreased[16,17]. Treatment with ascorbic acid, an antioxidant vitamin, caused a significant reduction of toxic effects of this pesticide. The administration of LCT in different periods of postnatal ontogenesis was also reported to enhance oxidative stress by a significant increase in MDA level and suppressed activity of antioxidant enzymes (SOD, CAT) in brain tissue[38]. The ability of LCT to induce a pronounced oxidative stress was also demonstrated in a vitro study[14, 18]. There is no clear information regarding the toxic effect of LCT on female rats. In our study we found that administration of LCT to rats resulted in a marked dose-dependent increase in the lipid peroxidation as indicated by the increase in the level of malondialdehyde (MDA), that may be due to LCT induced increased ROS level.

GSH, one of the most important biological molecules, play a key role in the detoxification of the reactive toxic metabolites. Decline in GSH levels in ovary after LCT treatment may be an indication of oxidative

stress, whereas GSH is utilized for the detoxification of reactive toxic substances. An increased level of GSSG also reflects the oxidative stress of ovary. Normal cellular functioning depends on a balance between ROS production and antioxidant defense mechanisms present in the cell. Antioxidant enzymes are considered to be a primary defense that prevents biological macromolecules from oxidative damage.

According to the results, the activities of SOD, CAT, glutathione peroxidase and glutathione reductase in ovaries of LCT treated rats were significantly decreased. There was no significant changes found in GST level in LCT treated low dose group but marked alteration has been found in LCT treated high dose group. These results suggested that LCT has the capability to induce free radicals and oxidative damage as evidenced by alterations in various antioxidant enzymes [39]. Reduction of antioxidant enzymes levels may be due to the direct effect on the enzymes against LCT-induced ROS generation.

Taurine administration reversed all these abnormalities of above mentioned ovarian parameters to a good extent. It diminished lipid peroxidation either by scavenging or quenching oxygen-derived free radicals, hydrogen peroxide or hypochlorous acid directly, or by binding free metal ion species like Fe^{2+} or Cu^{2+} by its sulfonic acid group. By decreasing carbonyl group production, taurine suggested to decrease enhanced oxidative damage[40, 41]. On the other hand, since cysteine is a precursor of taurine and GSH, taurine supplementation may cause enhancement in GSH levels by directing cysteine into the GSH synthesis pathway[41,42]. Therefore, increased GSH levels after taurine treatment may play an additional role in decreasing oxidative stress. The stimulatory effects of taurine on endogenous antioxidants were also established by other studies [43,44].

CONCLUSION

Considering the above results we may conclude that LCT has the potency to induce toxicity as well as oxidative stress in ovary; thereby alter normal estrous cycle and oxidative stress parameters. Administration of taurine attenuates the toxicity of LCT in ovary.

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