EFFECT OF LONG TERM EXPOSURE OF LOW DOSES OF LAMBDA-CYHALOTHIRIN ON THE LEVEL OF LIPID PEROXIDATION AND ANTI-OXIDANT ENZYMES OF THE PREGNANT RATS AND THEIR OFFSPRING

Lambda-cyhalothrin (LCT) is a pyrethroid insecticide class, which is widely used for pest control in agriculture, public health, home and garden. In the present study we investigated the effect of long term exposure of low doses of the LCT on the state of lipid peroxidation and antioxidant protection of pregnant rats and their offspring. It was revealed, that prolonged exposure of lambda-cyhalothrin leads to the development of oxidative stress in both of pregnant females and their offspring. The highest level of lipid peroxidation detected on 14-21 days of pregnancy, which was accompanied by a reduction in activity of antioxidant enzymes. In the offspring highest level of oxidative stress observed on 7-14 days of lactation. The degree of oxidative stress in offspring decreases as the cessation of receipt of a pesticide or its toxic metabolites in breast milk.

KADIR TUKHTAEV, SABIRJAN TULEMETOV, NARGIZA ZOKIROVA, NODIRBEK TUKHTAEV

Tashkent Medical Academy
Tashkent, Uzbekistan

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Introduction

Lambda-cyhalothrin (LCT) is a pyrethroid insecticide class, which is widely used for pest control in agriculture, public health, home and garden (Amweg and Weston, 2005). In agriculture, the objects of these actions are cotton, cereals, various vegetables and fruits with applications made to control aphids and other pests. Pyrethroids are an important tool in public health management, where applications are made to control cockroaches, mosquitoes, ticks and flies, which can act as disease vectors. Residential use of pyrethroid products has grown and continues to grow due to the suspension of organophosphorus products containing chlorpyrifos or diazinon (He, Troiano, Wang, and Goh, 2008). Widespread use of pyrethroids has also contributed to instability in the environment through hydrolysis, photo- and microbial degradation. Pyrethroids are axonic poisons that affect the nerve fiber by binding to a protein that regulates the voltage-gated sodium channel. Type II pyrethroids (e.g., LCT) can also affect chloride and calcium channels that are important for proper nerve function. Because of the lipophilic nature of pyrethroids, biological membranes and tissues readily absorb them. Specifically, LCT penetrates the insect cuticle, disrupting nerve conduction within minutes; this leads to cessation of feeding, loss of muscular control, paralysis, and eventual death (He et al., 2008).

Initially it was believed that pyrethroids have a strong toxic effect on insects in very small doses that are relatively harmless to vertebrates and mammals (Rundle and Forsyth, 1984). However, subsequent studies have shown that pyrethroids, including lambda-cyhalothrin, are quite toxic to mammals and humans (Shakoori, Aslam, Sabir, and Ali, 1992; Wolansky, Gennings, and Crofton 2006; Yousef, 2010). The degree of toxicity depends on dose, method and duration of exposure.

There are several reports of toxicity LCT to mammals and the ability of this pesticide to induce oxidative stress in vivo and in vitro (El-Demerdash, 2007; Fetoui et al., 2008; 2009; Abdallah, Fetoui, Fakhfakh, and Kèskes, 2012). However, information on the status of lipid peroxidation and antioxidant defense system during prolonged exposure to low doses of this pesticide is publically unavailable. It should be noted that in natural
conditions, the animals and humans exposed to repeated low-dose effects of this pesticide, and pregnant women and children in this plan represent a group at particular risk. For this reason in the present study we investigated the effect of long term exposure of low doses of the LCT on the state of lipid peroxidation and antioxidant protection of pregnant rats and their offspring.

**Material and methods**

Lambda-cyhalothrin as a 10% emulsion concentrate (trade name "Titan") received from the joint Uzbek-German Company "Euro-Team". Experiments were performed on white adult virgin female rats Wistar weighing 150-170 grams and sexually mature male rats were used for fertilization. All rats were kept under controlled temperature (22±3°C) and humidity (40-70%) with a 12 hour light-dark cycle. Animals were on a standard laboratory diet and given water *ad libitum*. Rats were acclimatized for one week prior to the start of experiment. Then the female rats were divided into two groups of 45 rats each. The first (treated) group of rats was administered *per os* through gavage diluted in saline lambda-cyhalothrin at the rate of 8 mg/kg/day. This corresponded to 1/100 of LD$_{50}$ of the drug. The second (control) group in the same way received the same volume (0.4 ml/rat/day) of sterile saline. The administration of the drug did not stop until the end of the experiments. On 31 day of experiments female rats of both groups were combined with male rats for fertilization. Pregnancy was monitored by the presence of sperm in vaginal smears. After becoming pregnant females separated from males and placed in separate cages for future research. The parts of both group pregnant females were sacrificed at 14 and 21 days of gestation (GD 14 and GD 21) under light ether anesthesia. Other rats were sacrificed in the same way at 14 and 21 days after birth (lactation day, LD 14 and LD 21). It should be noted that long-term administration of low doses of lambda-cyhalothrin did not lead to the appearance of overt symptoms of toxicity in experimental rats. Only a few rats were found mild lethargy and a slight decrease in motor activity. Offspring of females treated with LCT by the number and size did not significantly differ from controls. There are only a belated opening of the eyes and detachment of ears compared to control. Offspring from both groups of animals were sacrificed at 7, 14, 21 and 30 days (postnatal days, PND 7, PND 14, PND 21 and PND 30) after birth under light anesthesia with ether.

After sacrificing the liver was immediately removed, weighed and cleaned of extraneous tissue and rinsed with ice-cold saline solution. To obtain an extract of 1 g liver was homogenized in 10 ml of cold phosphate buffer solution (pH 7.4). The homogenate was centrifuged at 8000 rpm for 15 min at 4°C and the resulting supernatant was used for biochemical studies. Biochemical determination of the status of lipid peroxidation and antioxidant enzyme levels was carried out by known methods adopted in our laboratories (Karimov, Inoyatova, and Mukhamedova, 2004). Briefly, the level of maleic dialdehyde (MDA) was determined on the basis of the reaction with thiobarbituric acid (TBA) to form a colored complex (TBARS), which is then calculated by spectrophotometry and expressed as nmol /mg protein. The activity of superoxide dismutase (SOD) were determined spectrophotometrically using nitro blue tetrazolium as an indicator reagent and expressed as unite/min mg protein. In determining the activity of catalase (CAT) as a substrate using hydrogen peroxide, activity of CAT expressed as H$_2$O$_2$/min mg protein.

Calculation and statistical analysis was performed using the statistical package for Window’s. All data were represented as mean ± standard deviation (SD). Statistical significance between control and treated values were compared using Student's LSD test and P values less than 0.05 were considered significance.

**Results**

The findings showed that prolonged exposure to low doses of LST leads to significant induction of oxidative stress in the rat females (Figure 1). In treated female rats before fertilization MDA level was nearly three times higher than control values. Pregnancy
promoted to greater induction of oxidative stress. In control rats on days 14 and 21 there are only a slight increase of MDA levels compared with baseline data. At the same time in the treated females the level of MDA was in the same days, respectively, 3.6 and 3.4 times higher than control values (P <0.001).

**FIGURE 1. THE LEVEL OF LIPID PEROXIDATION (MDA) IN LIVER TISSUE DURING PREGNANCY AND LACTATION**

![Graph 1](image1)

**FIGURE 2. THE ACTIVITY OF ANTIOXIDANT ENZYME SUPEROXIDE DISMUTASE (SOD) IN LIVER TISSUE DURING PREGNANCY AND LACTATION**

![Graph 2](image2)

Note: * Differences were significant compared with controls. GD - Gestational days; LD - Lactational days

**FIGURE 3. THE ACTIVITY OF ANTIOXIDANT ENZYME CATALASE (CAT) IN LIVER TISSUE DURING PREGNANCY AND LACTATION**

![Graph 3](image3)

**FIGURE 4. THE LEVEL OF LIPID PEROXIDATION (MDA) IN LIVER TISSUE OF OFFSPRING IN THE DYNAMICS OF POSTNATAL DEVELOPMENT**

![Graph 4](image4)

Note: * Differences were significant compared with controls. GD - Gestational days; LD - Lactational days

After birth, levels of MDA, although in general somewhat lower, but still 2.7 - 3 times higher than control indexes. Somewhat different results were obtained in the study of antioxidant enzymes (Figures 2 and 3). The activity of SOD and CAT in the treated female rats prior to pregnancy was more than 2 times higher compared with controls. On day 14 of pregnancy was found tended to decrease the activity of both enzymes and on day 21 revealed their significant decrease as compared to control. After birth the activity of these enzymes increased again and on day 21 of lactation was more than 2 times higher than control indices. Prolonged exposure to low doses of LCT contributed to a significant induction of oxidative stress not only in treated mothers, but also in their offspring (Figure 4). The level of MDA progressively increased in the offspring, and its maximum value was observed at day 14 after birth. Then the MDA level gradually decreased and on day 30 was not significantly different from controls. The activity both of SOD and CAT on day 7 after birth was more than 2-fold higher than control values (Figures 5 and 6).
Induction of oxidative stress is one of the main mechanisms of the action of many pesticides (Abdollahi, Ranbar, Shadina, Nikfar, and Rezaie, 2004; Amin and Hashem, 2012). Oxidative stress induction involves an excessive production of reactive oxygen species (ROS or free radicals) resulting from impaired balance between the ROS generation and antioxidant defense capability. Antioxidant enzyme defense system includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and others that may protect the system from deleterious effects of oxygen free radicals. The damage of membrane lipids, protein and DNA is endpoint biomarker of oxidative stress inducing effect of many pesticides (Tuzmen, Candan, Kaya, and Demiryas, 2008).

Lambda- cyhalothrin in certain doses and conditions is also a potent inducer of oxidative stress (Piner and Uner, 2012). Fetoui et al. (2009; 2010) showed that LCT significantly increases the level of MDA in the liver and kidneys of rats, whereas the activity of antioxidant enzymes (SOD, CAT) decreased. Treatment with ascorbic acid resulted in a significant reduction of toxic effects of this pesticide. Ansari et al. (2012) found that the administration of LCT in different periods of postnatal ontogenesis causes enhanced oxidative stress, manifested by a significant increase in MDA level and suppressed the activity of antioxidant enzymes (SOD, CAT) in brain tissue. The ability of LCT to induce a pronounced oxidative stress was also demonstrated in vitro (El-Demerdash, 2007; Abdallah et al., 2012). The degree of oxidative stress is further enhanced if the LCT is used in combination with other pesticides (El-Demerdash, 2011; 2012). Our data showed that prolonged exposure to LCT (30 days before pregnancy, 21 days of gestation and 21 days after pregnancy) leads to a marked oxidative stress. We found that pregnancy promoted even greater induction of oxidative stress. The toxic effect of LCT on pregnant maternal organism was studied only in few papers (Ratnasooriya, Ratnayake, and Jayatunga, 2003). The authors believe that LCT is dangerous for pregnancy outcome (in terms of quantum of pregnancy, the number of uterine implants, implantation index and fetal death), while in the offspring do not show developmental defects. In their view, the
impact of LCT in the early stages of pregnancy can lead to risk of pregnancy. However, when exposed to LCT, we found no reduction in the number of offspring, the threat of miscarriage or other obvious signs of pregnancy disorders. Offspring of females treated with LCT by the number and size did not significantly differ from controls. There are only a belated opening of the eyes and detachment of ears compared to control. It is well known that the pregnant body is most sensitive to the effects of various xenobiotics (Agarwal et al., 2012). Our data showed that during pregnancy, despite high levels of MDA, the activity of SOD and CAT decreased and on day 21 of gestation revealed a significant decrease of as compared with controls. After birth, the activity of these enzymes again increased and on day 21 of lactation was more than 2-fold higher than control values. These data suggest that an additional toxic effects in pregnancy lead to depletion of reserves of antioxidant defense system. This in turn adversely affects the intrauterine and postnatal development of the fetus.

Several studies have shown that the use of ascorbic acid (Fetoui et al, 2009, 2010), alpha-tocopherol or vitamin E (Amin and Hashem, 2012) leads to a reduction in oxidative stress at exposure of LCT and restores the activity of enzymatic antioxidant defense systems. Recent studies have revealed that caffeic acid and quercetin are also able to protect against oxidative stress and genotoxic effect of LCT in vitro (Abdallah et al., 2012). Although we have not directly studied the effect of antioxidants during pregnancy in conditions exposure of LCT, these data suggest the feasibility of the use of antioxidants in pregnant women with possible risk of exposure of pesticides, especially LCT and its metabolites. This is especially true of well-known and readily available medicines such as ascorbic acid and alpha-tocopherol.

Early postnatal period is most vulnerable to the action of environmental hazards (Ahmed, Abd El-Tawab, and Ahmed, 2010; Ahmed, 2011). In the offspring from mothers with long-term exposure LCT, we also observed a significant induction of oxidative stress. The level of MDA progressively increased in the offspring, and its maximum value was observed at day 14 after birth. Then the MDA level gradually decreased and day 30 of postnatal period was not significantly different from controls. This means that oxidative stress, which arose as early as the embryonic period, continues to develop in postnatal life. The degree of oxidative stress decreases as the cessation of receipt of a pesticide or toxic metabolites in breast milk. This explains the decrease of MDA and SOD and CAT to 30 days after birth, when completely stopped the intake of LCT or its metabolites. These data suggest that an effective antioxidant therapy to pregnant women both during pregnancy and lactation may indirectly prevent or reduce the degree of oxidative stress in the offspring.

Conclusion

Prolonged exposure of low doses of lambda-cyhalothrin leads to the development of oxidative stress in pregnant females and their offspring. The highest level of lipid peroxidation detected during pregnancy, which was accompanied by a reduction in activity of antioxidant enzymes (SOD and CAT). In the offspring highest level of oxidative stress observed during lactation. The degree of oxidative stress in offspring decreases as the cessation of receipt of a pesticide or its toxic metabolites in breast milk.

References


