

Effects of Lambda cyhalothrin on Reproductive Characteristics in Pregnant Rabbit doe (*Oryctolagus cuniculus*)

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Abstract

Introduction: Lambda-cyhalothrin (LCT) is a manufactured pyrethroid insecticide, and has now become an environmental issue due to its excessive use in agriculture, livestock production, leather industry and shampoos. The present study was undertaken to evaluate the effects of LCT on reproductive characteristics in pregnant rabbit does.

Methods: Fifteen nulliparous and sexually mature rabbit does, aged 8 months and weighing 2.80-3.00 kg were divided into three groups of five animals, comparable in terms of body weight (bw). After mating, group T0 received distilled water, while groups T1 and T2 were administered doses of 4.16 and 8.32 mg/bw of LCT respectively. At 28th day post-coïtum, animals were sacrificed for evaluation of reproductive parameters. Data were subjected to one-way analysis of variance to observe significance difference against control.

Results: Results showed comparable ($P > 0.05$) values for reproductive organs weight no matter the organ nor the group of treatment considered. A dose-dependent decrease in serum progesterone concentration was noted; nevertheless, the difference was not significant ($P > 0.05$) among groups. Likewise, an insignificant ($P > 0.05$) decrease was observed in the number of implantation sites, live fetus, placenta and litter size; with an increase in the number of death fetuses, pre-implantation resorptions, post-implantation resorptions and total resorptions in lambda-cyhalothrin treated groups when compared with the control. A decrement was registered in fetus weight, lengths of the head, crown-rump, tail and total body length in the 2 groups treated with the pesticide as compared to the control group; though only significant ($P < 0.05$) in fetus weight.

Conclusion: The present study indicates that administration of lambda cyhalothrin at high doses exerts potentially adverse effects on the reproduction of rabbit does.

INTRODUCTION

Reproduction is the essential function that allows species continuity. It improves the productivity and perpetuates animal species. Its perturbation or dysfunction leads to negative consequences on the animal productivity [1]. Many factors (heat, feed, ageing, exposure to xenobiotics such as drugs, heavy metals and pesticides) can be responsible for this perturbation. One of the main mechanisms by which these factors alter reproduction is oxidative stress (OS). OS occurs

when the generation of reactive oxygen species (ROS) and other radical species exceed the scavenging capacity of anti-oxidants (AO) in an organism [2]. Pesticides (insecticides, herbicides and fungicides) constitute the major potential environmental hazard to humans and animals as these are present in the food chain. World Health Organization has prohibited the use of pesticides having acute toxicity, but pyrethroids use is extensive. The pyrethroid pesticides are

rapidly replacing other insecticides because they are known to be less harmful to mammals, birds and less toxic to the environment than other insecticides [3].

Pyrethroid pesticides are used worldwide as insecticides in pest control by disrupting the normal function of sodium channels. However, they have now become an environmental issue due to excessive use in agriculture, livestock production, leather industry and shampoos etc. In addition, pyrethroid pesticides have been found in environmental samples, such as water and sediments, food; and they can also be found in urine and breast milk under human samples [4]. Metabolites of pyrethroids have also been shown to exert adverse effects on different physiological functions in the body [5]. In connection to the exposure of this pesticide, it was reported that diseases such as reproductive disorders, cancer, neurological disorders, allergies, mental disorders could be connected [6] and immune and hormone systems of humans and animals [7]. Furthermore, oxidative stress effects of pyrethroid-induced toxicity have been reported by some investigators [8]. Lambda-cyhalothrin (LCT) is a manufactured pyrethroid insecticide that is being used in home pest control, agriculture, protection of food and disease vector control. In agriculture, it is used for cotton, cereals, various vegetables and fruits with applications made to control aphids, Colorado beetles and lepidopteran larvae [9].

However, subsequent studies have shown that lambda-cyhalothrin, are quite toxic to mammals and humans [10]. The degree of toxicity depends on dose, method and duration of exposure. There are several reports on the toxicity of LCT to mammals and the ability of this pesticide to induce oxidative stress in vivo and in vitro [11].

In addition to various clinical, hemato-biochemical, immunosuppressive and neuro-toxicological effects of pyrethroids, more danger has been suspected with respect to reproductive toxicity. Studies on reproduction reported male reproductive toxicity, that is decreased semen quality, weight of testes and epididymis in rabbits [10]; sperm abnormalities, reduced sperm count and motility in rats [12]; blocked spermatogenesis, damaged seminiferous tubules in animals exposed to LCT. However, reports on studies available are mostly on males or on Pyrethroids in general. Keeping in view the scarcity of information on LCT toxicity in females, this study was designed and executed to investigate the propensity of Lambda-cyhalothrin to prompt changes in the reproductive characteristics in pregnant does.

MATERIALS AND METHODS

Animal material

Healthy fifteen adult fertile rabbit-does (New Zealand breed) of 8 months old, weighing 2.8–3.0 kg, produced at Teaching and Research Farm of the University of Dschang were used. They were treated against external (exo) and internal (hemo) parasites by sub-cutaneous injection of an ivermectin solution (0.1 ml/kg bw), repeated after two weeks.

Lodging and feeding

Animals were housed in a cement block building with sheet metal roof, plastered and open 1/3 upper section. They were kept individually in wire cages (galvanized metal, 96 cm long, 40 cm wide, 15 cm high). Each cage was equipped with a feeder and a drinker (800 ml capacity). The building was previous-

ly disinfected with a solution of javel water and cresyl (1/2 l each mixed in 20 l water) which was sprayed in the building and in all the cages. Animals were introduced in the cages two weeks after disinfection. Throughout the trial period, animals received commercial complete feed from from SPC (*Société des Provenderies du Cameroun*) and water *ad libitum*. The chemical characteristics of this feed are summarized in Table 1.

Chemical characteristics	Values
Fats (% DM)	6.50
Calcium (% DM)	1.20
Crude fiber (% DM)	7.00
Crude protein (% DM)	16.00
Metabolisable energy (Kcal/kg DM)	2350.00
Humidity (% DM)	12.00
Vitamins	+
Oligo-elements	+
Antioxidants	+
Anti-moisture	+

DM: Dry Matter.

Preparation of lambda-cyhalothrin solutions

Doses of Lambda-cyhalothrin used were 0, 1/100 and 1/50 of the LD₅₀ (416 mg/kg body weight) reported in rabbit doe by Morgan and Osman, [13]. That is 0, 4.16 and 8.32 mg/kg body weight. Lambda-cyhalothrin solutions were prepared by diluting known volumes of KILLAM 15 EC (Lambda-cyhalothrin 15 g/L) from INTRADE LTD SAS- France, in distilled water in such a way to obtain final solutions at required concentrations.

Experimental design

Fifteen fertile rabbit-does were mated with untreated sexually mature males, with sex ratio 1:3 (1♂ for 3♀), and randomly divided into 3 groups of 5 rabbit-does each, comparable in terms of body weight (bw). The 3 groups were assigned different treatments for 28 days *post-coitum*.

Lambda-cyhalothrin was administered by gavage using a syringe of 2.5 ml. The animals of the control group (T0) received 0.5 ml/kg bw of distilled water daily. Groups T1 and T2 were given doses of 4.16 and 8.32 mg /kg bw respectively. At 28th day *post-coitum*, animals were sacrificed for evaluation of reproductive characteristics.

Reproductive characteristics

The abdominal cavity was opened and the uterus, ovaries and placenta were removed and weighed with the help of an electrical scale of 160 g capacity and 10⁻³g precision.

Number of foetuses, litter size, litter weight and kid body measurements

- The uterus was opened and the number of foetuses obtained by counting.
- Litter size was obtained by counting the number of kids

from each female;

- Kids were weighed after collection and lengths of fetus body, head, crown rump and tail were measured using a Vernier caliper.

Resorptions

The number of *corpus luteum* was counted on the ovaries. The uterus was steeped in a 2% sodium hydroxide solution for 10 minutes and the number of implantation sites was counted in order to determine the number of resorptions. The number of *corpus luteum* was compared to the number of implantation sites which permitted to determine the number of pre-implantation resorptions using the following formula:

Pre-implantation resorptions = number of *corpus luteum* – number of implantation sites

Post-implantation resorptions were early when only placenta tissues were visible and late when placenta tissues and embryony tissues were observed. The following formula was used:

Post-implantation resorptions = number of implantation sites – number of life foetuses

Hormonal analysis

Blood samples were obtained by cardiac puncture and collected in tubes free from anticoagulant for dosages. Progesterone was measured in serum using AccuDiag™ ELISA kits from OMEGA DIAGNOSTICS LTD (Scotland, England). Progesterone concentrations were obtained by projecting optical density of samples read on ELISA meter on calibration curves of progesterone constructed from standard concentrations provided by the kit.

Statistical Analysis

Data were submitted to one-way analysis of variance (ANOVA) to test the effects of lambda-cyhalothrin doses on studied parameters. The Duncan test was performed to separate means when there was a significant difference. The results were expressed as mean ± standard deviation and a limit of significance was fixed at $P < 0.05$. The software SPSS 20.0 was used for the analysis.

RESULTS

Relative weights of reproductive organs in pregnant does exposed to lambda cyhalothrin

The effects of lambda cyhalothrin on weights of some repro-

ductive organs in pregnant does are shown on Table 2. The weights of ovary, uterus and placentas tended to decrease in Lambda-cyhalothrin-treated does. Nevertheless, no significant ($P > 0.05$) difference was observed among treatments.

Progesterone concentration in pregnant does exposed to lambda cyhalothrin

Fig. 1 illustrates the serum concentration of progesterone in pregnant does exposed to lambda-cyhalothrin. A decrease in serum progesterone concentration was recorded in animals treated with lambda-cyhalothrin in comparison to those that received distilled water. But, no significant ($P > 0.05$) difference was recorded among groups.

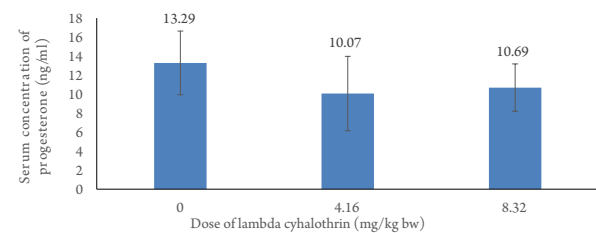


Figure 1: Serum concentration of progesterone in pregnant does exposed to lambda cyhalothrin.

Fetotoxicity in pregnant does exposed to lambda cyhalothrin

The effects of lambda-cyhalothrin on some reproductive traits are summarized on Table 3. It shows that, the number of implantation sites, live fetuses, placenta and litter size decreased in does treated with lambda-cyhalothrin with respect to the those given distilled water.

Meanwhile, an increment was observed in the number of death fetuses, pre-implantation resorptions, post-implantation resorptions and total resorptions in lambda-cyhalothrin-treated groups when compared with the control. Generally, a marked increase was observed in the group exposed to 8.32 of lambda-cyhalothrin with respect to the other groups. Nevertheless, no significant ($P > 0.05$) difference was registered among treatments.

Fetal measurements in pregnant does exposed to lambda cyhalothrin

Table 4 presents the effects of lambda-cyhalothrin on some fetal measurements. A decrement is registered in fetus weight, lengths of the head, crown-rump, tail and total body length in the 2 groups treated with the pesticide as compared to the control group; though the decrease was only significant ($P < 0.05$) for fetus weight.

Weight of reproductive organs	Doses of Lambda cyhalothrin (mg/kg bw)			P
	0 (n = 5)	4.16 (n = 5)	8.32 (n = 5)	
Ovary (g/100 g pc)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± .01	0.357
Empty uterus (g/100 g pc)	1.11 ± 0.27	1.04 ± 0.19	1.11 ± 0.24	0.870
Placenta (g/100 g pc)	0.20 ± 0.04	0.18 ± 0.02	0.16 ± 0.04	0.356

n: number of animals

Table 3: Effects of lambda cyhalothrin on some fertility traits in pregnant does

Fertility traits	Doses of Lambda cyhalothrin (mg/kg bw)			p
	0 (n = 5)	4.16 (n = 5)	8.32 (n = 5)	
Number of <i>corpus luteum</i>	8.00 ± 0.00	8.25±0.96	9.67 ± 1.53	0.165
Number of implantation sites	8.00 ± 0.00	7.75 ± 0.96	9.00 ± 2.00	0.439
Number of placenta	8.00 ± 0.00	7.75 ± 0.96	8.00 ± .64	0.969
Litter size	8.00 ± 0.00	7.75 ± 0.96	8.00 ± 2.64	0.969
Number of live foetus	8.00 ± 0.00	7.50 ± 1.00	7.67 ± 2.06	0.933
Number of death foetus	0.00 ± 0.00	0.25 ± 0.50	0.33 ± 0.58	0.653
Pre-implantation resorptions	0.00 ± .00	0.50 ± 1.00	0.67 ± 0.58	0.528
Post-implantation resorptions	0.00 ± 0.00	0.25 ± 0.50	1.00 ± 0.73	0.465
Total resorptions	0.00 ± 0.00	0.75 ± 0.46	1.67 ± 1.08	0.335

n: number of animals

Table 4: Effects of lambda cyhalothrin on some fetal measurements in does

Fetal measurements	Doses of lambda cyhalothrin (mg/kg bw)			p
	0 (n = 5)	4.16 (n = 5)	8.32 (n = 5)	
Weight (g)	29.75 ± 3.00 ^a	22.27 ± 4.32 ^b	24.67 ± 3.40 ^b	0.046
Head length (cm)	1.61 ± 0.15	1.60 ± 0.16	1.60 ± 0.11	0.940
crown-rump length(cm)	1.49 ± 0.14	1.40 ± 0.25	1.47 ± 0.10	0.401
Tail length (cm)	0.88 ± 0.07	0.95 ± 0.24	0.87 ± 0.09	0.144
Body length (cm)	8.33 ± 0.32	7.97 ± 0.86	8.19 ± 0.46	0.162

a, b: values affected with the same letter in the same line are not significantly different (p> 0.05). n: number of animals

DISCUSSION

Several studies have revealed that many pyrethroids and other insecticides develop a negative impact on the reproductive systems of humans and animals [6, 7]. Reproductive hazards encompass adverse health effects to the future mother and father (loss of libido, infertility, sterility) as well as to the developing offspring (abortion, fetal or prenatal death and teratogenesis) The reduced fertility in pyrethroid exposed female animals has also been discussed with relation to pathological effects on uterus, ovaries and hormonal influences [13].

The toxic effect of lambda-cyhalothrin (LCT) on pregnant maternal organism has been studied only in few papers [14]. Authors believe that LCT is dangerous for pregnancy outcome (in terms of quantum of pregnancy, the number of uterine implants, implantation index and foetal death), while 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 relevant developmental toxicity as offspring of females treated with LCT did not significantly differ from controls except in foetus weight.

The reduction in the size of head of pyrethroid exposed fetuses observed in the current work may reflect the reduction in the size of brain that occurs as a result of degenerative changes in neurons, which in turn might have occurred as a result of pyrethroid induced apoptosis.

Farag et al., [15] commented that maternal toxicity could be the trigger for the decreased pup weight gain and delayed development of physical features in the high dose group. The undersized progeny in this work might have developed alter-

ations in neuromuscular parameters afterwards justifying the significant weight decrease in lambda-cyhalothrin exposed fetuses. It has been quoted that the higher level of sensitivity of the neonatal mammals to pyrethroid toxicity might be due to partial ripeness of the enzymes which catalyze the pyrethroids metabolism in the liver of juveniles [15]. This may account for the general decline in fetal features though not significant.

The decrease in number of live fetuses could be a consequence of increase in death fetuses; and decrease in number of implantation sites may be due to slight oxidative damage caused by the pesticide in placenta which is susceptible to oxidative damage.

The results of this study are therefore in accordance with the work of Wolterink and Ray, [16], in which rats were given cyhalothrin at a dose of 0, 5, 10 or 15 mg/kg bw per day by gavage on days 6–15 of gestation. They observed no significant effect of treatment on the incidence of pregnancy, number, size, and sex of the fetuses and pre- and post-implantation loss. They equally carried out another study in female New Zealand White rabbits that received cyhalothrin at a dose of 0, 3, 10 or 30 mg/kg bw per day by gavage from day 6 to day 18 of gestation. No toxicologically relevant effects of treatment on incidence of pregnancy, gravid uterus weights, pre and post-implantation losses, number and sex of fetuses, or fetal crown/rump length were observed.

Still in line with this work, decreased weight of fetuses in cypermethrin exposed mammals [17] was observed.

Also, in the work carried out based on the available developmental toxicity studies in rats and rabbits, and the 3genera-

tion reproduction study in rats using cyhalothrin, by USEPA, [18], there was no increased quantitative or qualitative susceptibility to exposed foetuses. Likewise, WHO, [19] reported that treatment of low doses of cypermethrin (a synthetic pyrethroid pesticide) up to 500 mg/kg bw on female rats, mice and rabbits had no adverse effects on some reproductive parameters.

Nonetheless, the results of this study disagree with Rustamov and Abbasov, [20] who reported an increase in embryonic resorptions and [21] in which a decrease in mating index, implantation sites and fetuses recovered were reported in the dams allowed mating with male rats which were treated with different pyrethroids. Studies on cypermethrin exposure reported decreased number of fetuses, number of *corpus leutum* (CL), increased fetal mortality [17] in pregnant female rabbits; increased embryonic resorption, and decreased pre and post implantation sites, number of viable fetuses [17] in mammals; and increased number of dead pups in rats [15]. Organ weights are widely accepted in the evaluation of test article-associated toxicities. Reproductive organ weights in female rodents may have greater value in shorter duration toxicity studies (less than 6 months durations), because reproductive senescence in mature rats for example can begin as early as 6 months of age [22]. In both rodents and non-rodents, normal reproductive cycling and the effects of age cause notable inter-animal variation in uterine and ovarian weights.

In the current study, the observation could signify that the dose of lambda cyhalothrin administered did not have a pronounced effect on the anatomy of these organs. The current results are in line with Wolterink and Ray, [16] who showed that gravid uterus weights were comparable between the groups exposed to lambda cyhalothrin and control.

However, these results are different from those obtained by Das et al., [23], who reported a significant decline in the weights of ovary and uterus. In that study, decrease in ovarian index in case of cypermethrin treated rats compared to control may be due to decreased number of ovarian germ cells. Rat is a spontaneously ovulating species with estrous cycles and exhibits regular endocrine events. In that study, estrous cycle has been examined in the cypermethrin exposed female prepubertal rats as an indicator about the toxic effects of cypermethrin on the female reproductive function involving the component roles of hypothalamus, pituitary, ovary and uterus. The reduction in ovarian and uterine indices may be due to reduced anabolic role of estradiol on the weight of the ovary and uterus [24]. That decrease may equally be due to other toxic effects of cypermethrin on other systems of the animal.

Reproductive function is under the control of sex hormones. Several hormones produced by the hypothalamic-pituitary complex and the sex organs come into play in a precise and coordinated way in the control of folliculogenesis, oogenesis, the oestrous cycle, sexual behaviour and ovulation [25]. The decrease in progesterone concentration noted in this work may be attributed to oxidative damage caused by the pesticide in placenta which is susceptible to oxidative damage.

From these results it may be considered that lambda cyhalothrin affected the set-point that regulate the secretion and function of some reproductive hormones secreted from the ovary that control the estrous cycle function in female rabbit [26]. The reduction in the level of progesterone in lambda

cyhalothrin treated rabbits indicates that the pesticide may inhibit the function of ovary and placenta. The insignificant decrease in progesterone concentration is different from the report of Gill *et al.*, [27], who explored toxic effects of cypermethrin on bovine CLs *in vitro*, registering significantly decreased progesterone concentration and may be explained by the difference in doses administered.

From this study, it has been concluded that pre and post-implantation embryonic resorptions increase as indicated by the reduced number of implantation sites and average litter size, fetal weight and progesterone concentration depending upon the dose of exposure to the insecticide. Generally, the rates of anomalies though not significant are also increased with the increase of dose, indicating its adverse effects on reproductive efficiency and fertility in female rabbits. Therefore, over-dosage of lambda cyhalothrin must be avoided at any cost.

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ETHICAL STATEMENT

Experimental protocols used in this study were approved by the Ethical committee of the Department of Animal Science of the University of Dschang (ECDAS-UDs 23/02/2015/UDs/FASA/DSAES) and was in conformity with the internationally accepted standard ethical guide lines for laboratory animal use and care as described in the European Community guidelines; EEC Directive 86/609/EEC, of the 24th November 1986.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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This research received no external funding.

AUTHOR CONTRIBUTIONS

Conceptualization, design, data analysis and interpretation was done by M.T.M.A., N.F., V.B.N., and K.J. Data acquisition was done by M.T.M.A., V.B.N., C.M.M.M., T.H., D.M.N. and D.N.A.B.; Drafting of the article was done by M.T.M.A., V.B.N. and C.M.M.M.

DATA AVAILABILITY

The data sets used during the current study are available from the corresponding author upon reasonable request.

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