Food and Chemical Toxicology 48 (2010) 1152–1159

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Vitamin E modulates reproductive toxicity of pyrethroid lambda-cyhalothrin in male rabbits

Mokhtar I. Yousef *

Department of Home Economic, Faculty of Specific Education, Alexandria University, Alexandria, Egypt

ARTICLE INFO

Article history:
Received 23 November 2009
Accepted 1 February 2010

Keywords:
Reproductive toxicity
Lambda-cyhalothrin
Vitamin E
Rabbits
Testosterone
Free radicals
Enzymes

ABSTRACT

The objective of the current study was to analyze the reproductive toxicity caused by lambda-cyhalothrin (LCT) in male rabbits, and to evaluate the possible protective effect of vitamin E (Vit. E) as antioxidant. Animals were orally administered their respective doses of LCT every other day and given drinking water supplemented with vitamin E for 16 weeks. Results showed that semen quality was deteriorated following treatment with LCT. Also, testosterone levels, body weight (BW), feed intake (FI), and relative testes (RTW) and epididymis (REW) weights were significantly decreased. Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly increased in seminal plasma of rabbits treated with LCT compared with control. While, activities of glutathione *S*-transferase (GST), transaminases and acid phosphatase (AcP) were significantly decreased. Vitamin E alone significantly increased testosterone levels, BW, FI, RTW, REW, semen characteristics and seminal plasma enzymes, and decreased the levels of TBARS. Also, the present study showed that vitamin E might be effective against LCT-induced reproductive toxicity. It was suggested that LCT exerted a significant adverse effect on reproductive performance of male rabbits. Furthermore, vitamin E antagonized the toxic effects of LCT and improved semen quality of male rabbit.

1. Introduction

Several currently used pesticides, especially those having endocrine disruptive properties, are known to adversely impair reproductive competence of males under laboratory, field, clinical or occupational settings (Figa-Talamanca et al., 2001). Some of these agents are among the most commonly used pesticides/insecticides in developing countries including Egypt. In many countries lambda-cyhalothrin has been successfully used for control of infectious disease vectors, such as mosquitoes, triotomine bugs and other arthropods (Awumbila and Bokuma, 1994). Lambda-cyhalothrin is a potent, synthetic, type II pyrethroid. It is a stomach, contact and a residual insecticide, which acts as a neuropoison interfering in the ionic conductance of nerve membranes by prolonging the sodium current (Clark, 1997). In addition, pyrethroids increase the spontaneous release of neurotransmitters such as GABA, dopamine or noradrenaline (Clark, 1997), and may also acts as hormone disruptor (Garey et al., 1999). Collectively, the facts suggest that lambda-cyhalothrin (Ratnasooriya et al., 2002) may disrupt male reproductive function, but this has not been experimentally documented.

Synthetic pyrethroids (sumithrin, fenvalerate, d-trans allethrin, permethrin, cypermethrin and fenvalerate) have ability to disrupt estrogen signaling and caused alterations in reproduction (Yousef et al., 2003a). Also, exposure to lambda-cyhalothrin caused sexual dysfunction in male rats (Ratnasooriya et al., 2002). During pyrethroid metabolism, reactive oxygen species (ROS) were generated and caused oxidative stress in intoxicated animals (Kale et al., 1999; Yousef et al., 2003a, 2006). Antioxidants can protect against the damaging effect of oxygen species on sperm quality (Baker et al., 1996; Yousef et al., 2003a,b, 2004, 2005, 2006, 2007; Yousef, 2004; Yousef and Salama, 2009). Overproduction of ROS, however, can be detrimental to sperm, being associated with male infertility (Akiyama, 1999).

During the past few years, estimation of free radical generation and antioxidants defense has become an important aspect of investigation in mammals. Our recent studies were carried out to evaluate the potential role of antioxidant, such as, vitamin C, vitamin E, β-carotene, isoflavones, folic acid, propolis, curcumin and grape seed proanthocyanidin extract (Yousef, 2004; Yousef et al.,

Abbreviations: LCT, lambda-cyhalothrin; Vit. E, vitamin E; BW, body weight; FI, feed intake; RTW, relative testes weight; REW, relative epididymis weight; TBARS, thiobarbituric acid-reactive substances; GST, glutathione *S*-transferase; AST, aspartate transaminase; ALT, alanine transaminase; AcP, acid phosphatase; ROS, reactive oxygen species.

* Address: Department of Home Economic, Faculty of Specific Education, Alexandria University, 14 Mohamed Amin Shohaib Street, Moustafa Kamel, P.O. Box Roushdi, Alexandria 21529, Egypt. Tel.: +20 3 4252362/3 5454313; fax: +20 3 5442776.
E-mail address: yousefmokhtar@yahoo.com

0278-6915/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2010.02.002

2003a,b, 2004, 2005, 2006, 2007, 2008, 2009; Yousef and Salama, 2009) for the protection of cells against oxidative damage due to pesticides, heavy metals and chemotherapeutic agent toxicities.

Numerous antioxidants have proven beneficial in treating male infertility, such as vitamin C, vitamin E, glutathione, and coenzyme Q10 (Sinclair, 2000; Yousef et al., 2003a). Antioxidants can protect against the damaging effect of leukocyte-derived reactive oxygen species on sperm movement and may be of clinical value in assisted conception procedures (Baker et al., 1996). The production of reactive oxygen species (ROS) is a normal physiological event in various organs including the testis. Overproduction of ROS, however, can be detrimental to sperm, being associated with male infertility (Akiyama, 1999).

Vitamin E is believed to be the primary components of the antioxidant system of the spermatozoa (Surai et al., 1998), and is one of the major membrane protectants against ROS and lipid peroxidation (Akiyama, 1999). Supplemental vitamin E has been shown to increase total sperm output and sperm concentration (Brzezinska-Slebodzinska et al., 1995) in boars. Also, vitamin E is a naturally occurring antioxidant nutrient that plays important roles in animal health by inactivating harmful free radicals produced through normal cellular activity and from various stressors. The antioxidant function of vitamin E could, at least in part, enhance immunity by maintaining the functional and structural integrity of important immune cells (Chew, 1995). Because of the health problems induced by many environmental pollutants, much effort has been expended in evaluating the relative antioxidant potency of vitamin E. Therefore, the aim of this study was to assess (1) the potential impacts of lambda-cyhalothrin on body, testes and epididymis weights, plasma testosterone concentration and semen quality of male rabbits; (2) the role of vitamin E in alleviating the negative effects of lambda-cyhalothrin on reproductive characteristics.

2. Materials and methods

2.1. Chemicals

Lambda-cyhalothrin ($C_{23}H_{19}Cl–F_3NO_3$) was purchased from Kima Company, Egypt and vitamin E (Dietvit® E, 53% -tocopherol acetate), was manufactured by Codislait Sarl, 22120 Yffiniac, Neolait SA, France.

2.2. Animals and experimental design

Male New Zealand White rabbits (age of 7 months and initial weight of 2788 ± 32 gm) were used. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were individually housed in stainless steel cages. Feed and water were provided ad libitum. Rabbits were fed pellets consisted of 30% berseem (Trifolium alexandrinum) hay, 25% yellow corn, 26.2% wheat bran, 14% whole soybean meal, 3% molasses, 1% $CaCl_2$, 0.4% NaCl, 0.3% mixture of minerals and vitamins (0.01 gm/kg diet of vitamin E), and 0.1% methionine. The vitamin and mineral premix per kg contained the following vitamins: A-4,000,000 IU, D3–5000,000 IU, E-16,7 g, K-0.67 g, B1–0.67 g, B2–2 g, B6–0.67 g, B12–0.004 g, B5–16.7 g, Pantothinc acid-6.67 g, Biotein-0.07 g, Folic acid-1.67 g, Choline chloride-400 g; and minerals: Zn-23.3 g, Mn-10 g, Fe-25 g, Cu-1.67 g, I-0.25 g, Se-0.033 g, and Mg-133.4 g (Rabbit premix produced by Holland Feed Inter. Co.).The chemical analysis of the pellets (AOAC, 1990) showed that they contained 17.5% crude protein, 14.0% crude fiber, 2.7% crude fat and 2200 K cal. digestable energy/kg diet.

Twenty-four mature male rabbits were randomly divided into four equal groups of six rabbits each. Group 1 served as control. However, group 2 was given drinking water supplemented with vitamin E (2 mg/kg body weight). Group 3 was orally given lambda-cyhalothrin (LCT) (20 mg/kg body weight) every other day. Group 4 was given the combination of LCT and vitamin E. The experiment was continued for 16-week. The proper doses of lambda-cyhalothrin for each animal were placed into a syringe that was inserted orally with the help of plastic tube directly into the oropharyngeal region. The doses of the lambda-cyhalothrin and vitamin E were calculated according to the animal's body weight on the week before dosing. The tested doses for lambda-cyhalothrin and vitamin E were given every other day for 16-weeks.

2.3. Semen collection and characteristics

Daily feed intake and body weight were recorded weekly. Semen collection occurred weekly over the 16 weeks of the study, so 96 ejaculates obtained per experimental group. Ejaculates collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded after removal of the gel mass. A weak eosin solution was used for evaluation of sperm concentration by the improved Neubauer haemocytometer slide (GmbH+Co., Brandstwiete 4, 2000 Hamburg 11, Germany) (Smith and Mayer, 1955). Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma carried out immediately after collection according to Mann (1948). Assessment of live and abnormal spermatozoa was performed using an eosin–nigrosine blue staining mixture (Blom, 1950). The percentages of motile sperm were estimated by visual examination under low-power magnification (10×) using a phase-contrast microscope with heated stage. Total number of motile sperm calculated by multiplying percentage of motile sperm and total sperm outputs. Reaction time for the buck is calculated as the time needs for mounting a doe until complete ejaculation; it measured in seconds using a stopwatch. Initial hydrogen ion concentration (pH) of semen samples was determined immediately after collection using a pH cooperative paper (Universalindikator pH 0–14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) parameter was also calculated as (total sperm output × motility (%) × normal morphology (%)) (Correa and Zavos, 1996).

2.4. Blood collection and testosterone determination

Blood samples were collected from the ear vein of each buck every other week and placed immediately on ice in heparinized tubes. Plasma was collected from blood by centrifuged at 860*g* for 20 min and stored at −60 °C. Testosterone concentration in plasma was measured by simple solid phase enzyme immunoassay utilizing horseradish peroxidase as a tracer (Equipar, via G. Ferrari, Saronno, Italy). Intra and interassay coefficient of variations were 3.9% and 6.2%, respectively. All rabbits were euthanized at the end of the experimental period (16 week). Weight of testis and epididymis was recorded.

2.5. Seminal plasma collection and Biochemical parameters

Seminal plasma was obtained by centrifugation of semen samples at 860 Xg for 20 min at 4 °C, and was stored at −60 °C until analysis. The activities of aspartate transaminase (AST; EC 2.6.1.1) and alanine transaminase (ALT; EC 2.6.1.2) activities were determined with kits from SENTINEL CH. (via principle Eugenio 5–20155 MILAN, Italy). The method of Moss (1984) was used to assay the activity of acid phosphatase (AcP; EC 3.1.3.2). *p*-Nitrophenyl phosphate is hydrolyzed in acid pH medium by the action of acid phosphatase. Liberated *p*-nitrophenyl is spectrophtometrically quantified. Seminal plasma glutathione *S*-transferase (GST; EC 2.5.1.18) activity was determined according to Habig et al. (1974), using para-nitrobenzyl-chloride as a substrate. Thiobarbituric acid-reactive substances (TBARS) were measured in seminal plasma at 532 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 M-1Cm-1 was used for calculation (Tappel and Zalkin, 1959).

2.6. Statistical analysis

Data were analyzed as a randomized design (Steel and Torrie, 1981) using the General Linear Model procedure of SAS (1986). Dunnett post hoc analysis was used to compare means of treatment groups against the control. P values < 0.05 were accepted as significant.

3. Results

The present study showed that body weight (BW), relative weights of testes and epididymis, feed intake and plasma testosterone concentration were significantly ($P < 0.05$) decreased in rabbits treated with lambda-cyhalothrin as compared to either control or vitamin E-treated groups (Table 1). Vitamin E alone caused an increase ($P < 0.05$) in BW, relative weight of testes and epididymis, feed intake and plasma testosterone concentration as compared to control animals. In addition, co-treatment with vitamin E alleviated the toxicity of lambda-cyhalothrin with respect to the various tested parameters (Table 1).

Treatment with lambda-cyhalothrin decreased ($P < 0.05$) semen ejaculate volume (EV), packed sperm volume (PSV), sperm concentration, total sperm output (TSO), sperm motility (%), total motile sperm per ejaculate (TMS), total functional sperm fraction (TFSF), normal sperm, initial fructose and libido (by decreasing