Libyan Journal of Basic Sciences (LJBS)
Vol: 18, No: 1 , P:106-117 , December. 2022 https://ljbs.omu.edu.ly/eISSN 2707-6261

Deltamethrin -induced deterioration in reproductive performance of male rabbits: Protective role of vitamin B9

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DOI: https://doi.org/10.54172/esmsc072

Abstract

Deltamethrin (DLM) is a synthetic pyrethroid insecticide used worldwide in agriculture, home pest control, protection of foodstuff and disease vector control. Vitamin B9 referred to folic acid identified as a member of co-factors implicated in the metabolism and cellular processes of single carbon such as biosynthesizes of purine, thymidylate, and methionine. Therefore, this study aimed at elucidating the protective effects of V.B9 against the reproductive toxicity of DLM in male rabbits. Five rabbits per group were assigned to 1 of 4 treatment groups: control; DLM (1.28 mg/kg); V.B9 (5mg/kg) DLM (1.28 mg/kg bw) plus V.B9 (5 mg/kg bw). Results showed that live body weight (LBW), testes relative weight (RTW), and serum testosterone were significantly reduced ($P<0.05$) by treatment with DLM, also decreased ($P<0.05$) ejaculate volume, sperm concentration, total sperm output, sperm motility index, semen initial fructose concentration and TBARS. The negative effects of DLM on semen characteristics were dose-dependent. Treatment with V.B9 increased ($P<0.05$) LBW, RTW and serum testosterone concentration, improved semen characteristics, and alleviated the negative effects of DLM treatment increased ($P<0.05$) the numbers of abnormal and dead sperms in a dose-dependent manner. Results demonstrated the beneficial influences of V.B9 in reducing the negative effects of DLM on the production and reproduction of male rabbits.

Keywords: Deltamethrin, reproductive performance, vitamin B9 and rabbits.

Introduction

Deltamethrin (DLM) known insecticides and is widely used to control a broad spectrum of ecto-parasites (i.e. lice, flies and ticks) to protect crops, fruits, vegetables and fish from pests and parasites in terrestrial and aquaculture animal industries (*1*). Synthetic pyrethroids have high insecticidal potency and low toxicity to birds and mammals (*2*)**.** In rabbits exposed to DLM, a decrease in libido, ejaculate volume and sperm concentration was noted along with an increase in the percentage of dead spermatozoa at 1/100th the LD50, but the actual doses were not stated (*3*)**.** In a rat study, oral administration of DLM for 65 consecutive days decreased the conception rate in non-treated females that were mated with treated males with decreases in sperm concentration noted at the low dose of 1 mg/kg (39%) and 2 mg/kg (55%), respectively (*4*). The decrease in live sperm and plasma testosterone levels continued and was noted 21 days after administration of the chemical was stopped, along with degenerative changes in testicular and accessory gland structures. Intraperitoneal injection of DLM to male rats at 1mg/kg was shown to induce testicular apoptosis (*5*) and in another study in utero and lactational exposure to DLM induced subtle changes in reproductive behavior and physiology of male offspring (reduction in the number of animals with ejaculate) along with a decrease in testicular and epididymal absolute weights and the diameter of seminiferous tubules in the highest dose group of DLM (4.0 mg/kg). Subcutaneous exposure to DLM at doses as low as 0.003 mg/kg-day for a period of 30, 45 or 60 days produced an arrest of spermatogenesis, and a significant decrease ($p \leq 0.05$) in plasma FSH concentration compared to controls after 45 and 60 days, but not after 30 days, suggesting the hormonal system is targeted by DLM (*6*). Additionally in mice, oral administration at levels as low as 5 mg/kg-day of DLM alone or DLM and dimethoate administered together resulted in significantly decreased sperm count, motility and viability and a significantly increased percentage of morphologically-abnormal spermatozoa compared with the controls (*7*). Overall, there is evidence from a number of studies for a decrease in sperm count and increase in dead spermatozoa in mice, rats and rabbits at relatively low doses of DLM via several routes of exposure. The mode of action may be via altered hormonal levels. Vitamin B9 which also referred to folic acid identified as a member of co-factors implicated in Metabolism and cellular processes of

single carbon such as biosynthesizes of purine, thymidylate, and methionine (*8*). It is a body coenzyme that acts specifically exhibits antioxidant impact, erythropoiesis and synthesis of DNA (*9*), metabolism of DNA, amino acid (*10*), sperm formation (*11*) and male fertility in general (*12*). Folate depletion is connected with male fertility reduction indicators. Reduction of its level in seminal plasma, for example, it's associated with reduced sperm quality and elevated disruption human's sperm DNA (*13*). An inverse relation between the total administrated in day of folate was also observed with production of aneuploid sperm in humans (*14*). For DNA, RNA conversion, protein synthesis, folate generally found in vegetables with green leaf is essential. Since the synthesis of DNA is a primary part of spermatogenesis (*15*). It is necessary to have sperm with good movement and high activity. Just active sperm may pass through the genital tract, and movement is compromised with metabolism, dietary, and climate variables (*16*). Reproductive efficiency is also impaired by oxidative stress (*17*). Therefore, this study aimed at elucidating the protective effects of V.B9 against reproductive toxicity of DLM in male rabbits.

Materials and Methods

In this study DLM product (Butox) 50 mg/ml and Vitamin B9 were supplied from Pharaonia Pharmaceuticals, Al-Ajyal, Al-Bayda City. Mature male New Zealand white rabbits (age of 6 months and initial weight of (1892± 50.79 Kg) were used. Animals were individually housed in cages and weighed weekly throughout12- week's experimental period. Twenty mature male rabbits were randomly divided into four equal groups (each five rabbits) as follows:- Group I: Rabbits were used as control daily for 12 weeks. Group II: Rabbits were treated with V.B9 as given daily by gavage at a dose of 5 mg/kg B.W for 12 successive weeks (*18*). Group III: Rabbits were treated daily with DLM by gavage at a dose of 1.28 mg/kg B.W/day (*18*). Group IV: Rabbits were given with DLM daily at a dose of 1.28 mg/kg B.W./day by gavage like group III and given the V.B9 concurrently daily at a dose of 5 mg/kg B.W./day by gavage like group II for 12 successive weeks.

Body weight of each animal was recorded weekly throughout the 12-week of the experimental period. The weight measurements were carried out in the morning before access to feed and water. At the end of treatment period, all animals of each group were slaughtered. Weights of testis were

also recorded. Blood samples were collected from the ear vein of all animals every other week throughout the 6-weeks experimental period. The blood samples were collected in tube containing heparin to obtain plasma.

Characteristics of semen:

Semen collection was done weekly and continued throughout the 12-weeks experimental period, so 60 ejaculates obtained per treatment. Ejaculates were collected using an artificial vagina and a teaser doe. The volume of each ejaculate was recorded (using a graduated collection tube) 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, and Germany) (*19*).

Total sperm output calculated by multiplying semen ejaculate volume and semen concentration. Determination of initial fructose concentration in seminal plasma was determined immediately after semen collection (*20*). Assessments of dead and normal spermatozoa were performed using an eosin-nigrosine blue staining mixture (*21*). The percentages of motile sperm were estimated by visual examination under low-power magnification (10^{x}) using light microscope. Total number of motile sperm was calculated by multiplying the percentage of motile sperm and total sperm output. Reaction time was determined as the moment of subjecting a doe to the buck until the completion of erection; it was measured in seconds. Initial hydrogen ion concentration (pH) was determined immediately after collection using pH cooperative paper (Universalindikator pH 0-14 Merck, Merck KgaA, 64271 Darmstadt, Germany). Packed sperm volume (PSV) was recorded. Total functional sperm fraction (TFSF) was calculated as the product of total sperm output (TSO), sperm motility (%), and normal morphology (%) (*22*).

Statistical analysis

Where applicable, statistical analysis was carried out in Minitab software (version17) statistical significance was assessed using ANOVA analysis with Tukey multiple comparisons Test after detection normal distribution to the information and suitable $P < 0.05$ consider critical.

Results

Body weight (BW) relative weight of testes and testosterone were significantly ($P < 0.05$) decreased in rabbits treated with DLM compared to control animals (Table 1). Results obtained showed that DLM significantly (P

< 0.05) decreased libido (by increasing the reaction time), ejaculate volume, sperm concentration, total sperm output, sperm motility (%), total motile sperm per ejaculate (TMS), packed sperm volume (PSV), total functional sperm fraction (TFSF), normal and live sperm and semen initial fructose. While initial hydrogen ion concentration (pH) and dead and abnormal sperm were increased (P < 0.05). Concentrations of thiobarbituric acid-reactive substances (TBARS) were significantly (P < 0.05) increased in plasma of rabbits treated with DLM compared with control (Table 2). Vitamin B9 alone significantly (P<0.05) increased on all parameters, While TBARS decreased. The presence of vitamin B9 with DLM caused significant increase in the reduction of all parameters and this means that vitamin B9 alleviated its toxicity.

Table 1: The overall means (±SEM) of BW, RTW, blood plasma testosterone concentration and TBARS in plasma and testes during treatment of male rabbits with DLM, V.B9 and their combination.

Parameters	***Groups***			
	Control	***V.B9***	***DLM***	***DLM+V.B9***
BW (Kg)	2.268 ± 0.042^{ab}	2.400 ± 0.057^{a}	1.904 ± 0.029^{C}	2.156 ± 0.027^{b}
RTW (g/100 g BW)	6.06 ± 1.10^{a}	4.48 ± 1.13^{a}	7.13 ± 0.65^{a}	4.690 ± 0.554^{a}
Testosterone (ng/mL)	1.608 ± 0.039^{b}	1.793 ± 0.040^{a}	1.298 ± 0.074^{c}	1.623 ± 0.087^{b}
TBARS (nmol/ml)	2.680 ± 0.036^{b}	2.360 ± 0.063^{c}	3.128 ± 0.094^{a}	2.578 ± 0.036^{b}
TBARS (nmol/g tissue)	14.3 ± 1.88^{bc}	10.3 ± 0.13^{c}	26.6 ± 0.69^{a}	15.8 ± 1.59^{b}

^{abc} Within row, means with different superscript letters differ significantly ($p < 0.05$).

Table 2: The overall means (±SE) of semen characteristics during treatment of male rabbits with V.B9, DLM, and their combination

Parameters	***Groups***			
	Control	***V.B9***	***DLM***	***DLM+V.B9***
Ejaculate volume (ml)	0.49± 0.02^{a}	0.57± 0.02^{a}	0.30.± 0.02^{a}	0.45± 0.02^{a}
PH	6.63±0.02^{bc}	6.52±0.044^{c}	6.82±0.03 ^{a}	6.70±0.03 ^{ab}
Reaction time (s)	2.98±0.10^{b}	3.03±0.15^{b}	4.13 ±0.20 ^{a}	3.23±0.11^{b}
Packed sperm volume (%)	14.9±0.2 ^{b}	17.2±0.4^{a}	12.54±0.2^{d}	13.6±0.3^{c}
Sperm concentration (×10^{6} ml^{-1})	254±4.4^{b}	309± 7.0^{a}	219± 4.6 ^{c}	247±4.3^{b}
Total motile sperm (×10^{6})	69±3.1^{b}	112±6.1^{a}	42.5±3.2^{b}	63±2.9^{b}
Live sperm (%)	69.1± 0.8^{b}	76.3±1.6^{a}	58.2±1.1^{c}	67.0±0.7^{b}
Dead sperm (%)	27.8± 0.9^{b}	20.4± 1.6^{c}	38.9± 1.1^{a}	29.7± 0.7^{b}
Normal sperm (%)	82.4± 03 ^{b}	86.1± 0.4^{a}	78.4±0.7 ^{c}	80.2±0.3 ^{b}
Abnormal (%)	20±0.3 ^{b}	13±0.4 ^{c}	21±0.7 ^{a}	18±0.3 ^{b}
Total functional sperm fraction (×10^{6})	61±2.7 ^{b}	102±5.7^{a}	38±2.7^{c}	55±2.5^{b}
Initial fructose (mg/dl)	257± 3.9 ^{b}	277± 3.7^{a}	201 ±5.9^{d}	222±3.5^{c}

^{abc} Within row, means with different superscript letters differ significantly ($p < 0.05$).

Discussion

The present results indicate that treatment with DLM caused no significant change in body weight (BW) and increase weight of testes. The reduction in BW of the DLM treated rabbits is in agreement with (*3*). Also, monitoring of organ weight is an important parameter for the toxicological studies (*23*). In the present study, testes weight increased significantly due to administration of doses 1.28 g/kg BW of DLM as compared to control group. Testes are associated with metabolism and excretion toxicants including pesticides. Increase in weight of these organs may be a compensatory response to increase the functional demand for metabolizing and excreting higher dose of DLM. Study found that folic acid administration at different levels (0.01 or 0.5 g/l) in drinking water of rabbits had no significant effect on final body weight, while water intake significantly increased in treated compared with control groups (*24*). In broilers, reported that body weight was not affected by folic acid

administration (*25*). The confliction in these results may be attributed to dose or method of treatment and/or species of treated animal. The induction in the levels of TBARS (the marker of extent of lipid peroxidation) in plasma is in agreement with other finding (*26, 27*). Antioxidants are known to reduce oxidative-radical-induced reactions**.** The present results showed that treatment with folic acid alone caused decreases in the levels of TBARS in plasma. These results are in agreement with the findings obtained by (*28*), they reported that folic acid inhibited microsomal lipid peroxidation. Because folic acid is a water soluble molecule, the distribution of folic acid is expected to be much lower in lipid phase, and the maximum part of it remains in the aqueous phase. Epidemiological studies have shown that folate supplements can significantly reduce the risk of pancreatic cancer and breast cancer (*29*).

One other possible pathway is that folic acid may effectively inhibit NADPH oxidase mediated superoxide production leading to reduction of lipid peroxidation and subsequent decrease in MDA level (*30*). Also, previous studies demonstrated that semen of different animal species was adversely affected by DLM. Extensive use of environmental chemicals including pesticides without control is considered a potent hazard for serious environmental pollution and health threats including interference with male reproductive system which may impair male fertility (*31, 32*). Previously were studied the effects of DLM (5 mg/kg-day), dimethoate (5, 15 and 28 mg/kg-day) and a mixture of the two pesticides (5 mg/kg-day) on male reproduction in mice (*33, 34*).

After treatment, all male mice were weighed and killed with diethyl ether. Testes and epididymides were weighed and spermatozoa obtained were evaluated for percent motility and sperm content. Percent motility was determined by progressive and non-progressive movements of spermatozoa, and sperm count was determined. A significantly decreased sperm count, motility and viability and significantly increased percent morphologically abnormal spermatozoa compared with the controls was noted in the group exposed to dose levels of 5 mg/kg-day DLM and the group exposed to the mixture of dimethoate + DLM (5 mg/kg-day) (*33*). Result showed improving in the sperm quality after folic intake as significant ($P<0.05$) increased sperm motility, forward movement, and considerable decrease in abnormal movements like backward, vibration, and circulation also substantial ($P<0.05$) improved in sperm quality as a decrease in the dead and

deformity sperms percentage in comparison with control animals. And the discovery of a result agreement with (*34*) that demonstrated a substantial improvement in the semen production and fertilizing potential of rabbit buck spermatozoa. Following treatment of rabbit bucks with folic acid in drinking water.

In conclusion, the measured parameters could be used as bioindicators for the negative effect and reproductive toxicity of the exposure to DLM in adult rabbits. Using vitamin B9 combination with DLM minimized and alleviated the hazardous effects of DLM on most of the tested parameters and this may be attributed to the vital role of vitamin B9as antioxidant. In addition, treatment with vitamin B9 alone reduced the generation of free radicals and caused an improvement in the productive and reproductive performance of male rabbits.

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تدهور الأداء التناسلي لذكورالارانب بسبب الدلتامثرين و الدور الوقائي لفيتامين ب 9

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المستخلص العربي

دلتامثرين (**DLM**) هو مبيد حشري بيرثرويد اصطناعي يستخدم في جميع أنحاء العالم في الزراعة ومكافحة الآفات المنزلية وحماية المواد الغذائية ومكافحة ناقلات الأمراض. فيتامين ب 9 يشار اليه حمض الفوليك الذي تم تحديده كعضو في العوامل المشتركة المتورطة في التمثيل الغذائي والعمليات الخلوية للكربون الأحادي مثل التخليق الحيوي للبيورين والثيميديلات والميثيونين. لذلك هدفت هذه الدراسة إلى توضيح التأثيرات الوقائية لـفيتامين ب ضد السمية الإنجابية للـ **DLM** في ذكور الأرانب. تم تخصيص خمسة أرانب لكل مجموعة لواحدة من أربع مجموعات علاجية: مجموعة التحكم. **DLM (1.28** مجم / كجم) ؛ فيتامين ب 9 بجرعة **5** ملجم / كجم) **DLM (1.28** ملجم / كجم من وزن الجسم) زائد فيتامين ب 9 بجرعة 5 ملجم / كجم من وزن الجسم). أظهرت النتائج أن وزن الجسم الحي (**LBW**) والوزن النسبي للخصيتين (**RTW**) وهرمون التستوستيرون في الدم انخفض معنوياً (**P <0.05**) عن طريق العلاج بـ **DLM** ، كما انخفض (**P <0.05**) حجم السائل المنوي ، تركيز الحيوانات المنوية ، إجمالي إنتاج الحيوانات المنوية ، مؤشر حركة الحيوانات المنوية ، تركيز الفركتوز الأولي للسائل المنوي و **TBARS**. كانت الآثار السلبية للـ **DLM** على خصائص السائل المنوي تعتمد على الجرعة. أدى العلاج باستخدام **VB9** إلى زيادة (**LBW P <0.05)** و **RTW** وتركيز هرمون التستوستيرون في الدم ، وتحسين خصائص السائل المنوي ، وتخفيف الآثار السلبية لعلاج **DLM (P <0.05**) زيادة عدد الحيوانات المنوية غير الطبيعية والميتة بطريقة تعتمد على الجرعة . أظهرت النتائج التأثيرات المفيدة لـ **V.B9** في تقليل الآثار السلبية للـ **DLM** على إنتاج وتكاثر ذكور الأرانب.

الكلمات المفتاحية: الدلتامثرين ، الأداء التناسلي ، فيتامين ب 9 والأرانب.