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Pituitary-gonadal Hormones and Sperm Quality of Male Rats Treated with *Walterinnesia aegyptia* and *Bitis arietans* Venoms

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Abstract. Snake envenoming is a public health concern in Saudi Arabia. The effect of snake venoms, *Walterinnesia aegyptia* (*W. aegyptia*) and *Bitis arietans* (*B. arietans*) ($\frac{1}{10}$, $\frac{1}{5}$ and $\frac{1}{2}$ of LD_{50}) on concentrations of plasma testosterone, LH and FSH beside sperm analysis were studied at different time intervals.

The levels of plasma testosterone and LH hormones were not changed significantly in the rats injected with $\frac{1}{10}$ LD_{50} of *W. aegyptia* venom after one week only. FSH showed a significant decrease in the rats treated with the two venoms separately at the same previous dose after one week. The three tested hormones decreased significantly in the rats injected with $\frac{1}{5}$ or $\frac{1}{2}$ of LD_{50} *W. aegyptia* and *B. arietans* venoms.

The treatment of rats with $\frac{1}{10}$ LD_{50} of *B. arietans* venom has no significant influence on sperm quality and count, while the sperm motility decreased significantly and sperm abnormalities increased in the rats received $\frac{1}{10}$ LD_{50} of *W. aegyptia* venom. The sperm motility and abnormalities were affected significantly by $\frac{1}{5}$ and $\frac{1}{2}$ LD_{50} of both *W. aegyptia* and *B. arietans* venoms. The sperm count per epididymis did not change significantly in the rats given $\frac{1}{10}$ and $\frac{1}{5}$ of LD_{50} of *B. arietans* venom, while it lowered significantly in the rats injected with $\frac{1}{2}$ LD_{50} of the same venom after three weeks. Moreover, the sperm count lowered significantly in the rats injected with $\frac{1}{5}$ of LD_{50} of *W. aegyptia* venom after three and four weeks. The rats treated with $\frac{1}{10}$ LD_{50} of *W. aegyptia* showed no significant difference in the sperm count. In conclusion, the two venoms affected the testicular-pituitary hormones and sperm characteristics in dose and time dependent.

Keywords: Saudi Arabia, Hormone**,** Sperm**,** Venom, *W. aegyptia*, *B. arietans*.

Introduction

Snake venoms were reported to have various neurotoxic and hematological effects in both humans and animals [1]. The hematological effects include mainly hemorrhage, coagulation disturbances, hemolysis and general cardiovascular disturbances [2]. Other complicated symptoms related to physiological and metabolic changes were also reported [3]. It was reported that *Echis coloratus* venom caused severe hyperglycemia, a decrease in serum total lipids, elevation of AST and LDH, a decrease in the total protein of liver and the accumulation of glycogen in the liver [4]. Tissue damage due to envenomation is found to occur in many internal organs including the brain, lungs, heart, kidney, liver and endocrine glands [5].

It was reported that the scorpion venom given to rats was capable of inducing degenerative changes in seminiferous tubules, resulting in necrozoospermia [6]. Snake envenomation caused pituitary necrosis [7]. The venom of scorpion, *Tityus* sp., induced alterations in spermatogenesis, Sertoli cell vacuolation, spermatocyte arrest and the congestion of interstitial tissue [8]. The effects of snake venoms on serum hormonal levels have been reported [9]. Therefore, the present study investigates acute and chronic effects of the venoms of *Walterinnesia aegyptia* and *Bitis arietans* on pituitary-gonadal hormones of male rats, beside sperm quality.

Material and Methods

Venoms

Walterinnesia aegypyia (W. aegyptia) and *Bitis arietans (B. arietans)* venoms were milked at the Zoology Department, College of Science, King Saud University. The venoms were lyophilized and stored in desiccators at 4°C in the dark. LD_{50} (i. p.) of the venoms was determined [10] and was found to be 0.50 mg/kg for *W. aegyptia* and 1.30 mg/kg for *B. arietans*.

Animals

A total number of 210 male albino rats *Rattus rattus* (150-200 g, 4 months) was obtained from the animal house of College of Pharmacy, King Saud University. The first group of animals (n=30) served as the control and was injected (i.p.) with saline vehicle only. The remaining animals were divided into six equal groups (30 rats in each) and were injected with a single dose (i.p.) of $\frac{1}{10}$, $\frac{1}{5}$ and $\frac{1}{2}$ of LD_{50} of each venom according to the following table:

Venom dose	*W. aegyptia*	*B. arietans*
$\frac{1}{10}$ of LD_{50}	0.05 mg/Kg	0.13 mg/kg
$\frac{1}{5}$ of LD_{50}	0.10 mg/kg	0.26 mg/kg
$\frac{1}{2}$ of LD_{50}	0.25 mg/kg	0.65 mg/kg

All the animals treated with ½ of LD_{50} of *W. aegyptia* venom died after 10 days from injection. All the parameters were evaluated after 2 hours, 1 week, 2 weeks, 3 weeks and 4 weeks post-injection.

Methods

Blood samples were collected from the orbital sinus of the rats representing all groups at the different time intervals. Plasma was separated and kept at a temperature of (-21°C) till the assay. Plasma Testosterone, FSH and LH levels were assayed by enzyme linked immuno-sorbent assay (ELISA) method using the kits of DRG, Germany. The hormone kits are based on the principle of competitive binding. The microtiter wells are coated with an antibody. The sample hormone competes with a hormone horseradish peroxidase conjugate for binding to the coated wells. After incubation, the unbound conjugate is washing off. The amount of peroxidase conjugate is reverse proportional to the concentration of hormone in the sample. After the addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of hormone in the sample.

An evaluation of spermatozoa was determined; one epididymis was removed after sacrificing each rat at the different time intervals. It was cleaned from fats, dissected in 20 ml of normal saline (0.9% NaCl) and incubated at 37°C for 30 minutes. The percentages of sperm motility and abnormalities were determined as well as sperm count was carried out using haemocytometer. We observed 300 spermatozoa for each sample. The classification of individual spermatozoa was normal, head abnormalities and tail abnormalities. The percentage of total sperm abnormalities was calculated [11].

Statistical analysis

Statistical differences were calculated by using the student T-test [12].

Results

Testosterone levels

It is observed that the testosterone level of rats injected with 1/10 of LD_{50} of *W. aegyptia* decreased significantly ($p<0.01$) after 1 and 2 weeks (Fig. 1). Similar results were recorded in the case of rats injected with ⅕ of LD_{50} at the same intervals after 3 weeks. The testosterone level lowered significantly ($p<0.01$) in the animals administered the venom (i.p.) at the dose of ½ of LD_{50} after 2 hours and 1 week, and they died after that.

The testosterone level showed a significant decrease ($p<0.01$) in the animals injected with ⅕ and ½ of LD_{50} of *B. arietans* venom at all time intervals except the last one. The hormone level did not change significantly in the rats received 1/10 LD_{50} of *B. arietans* venom.

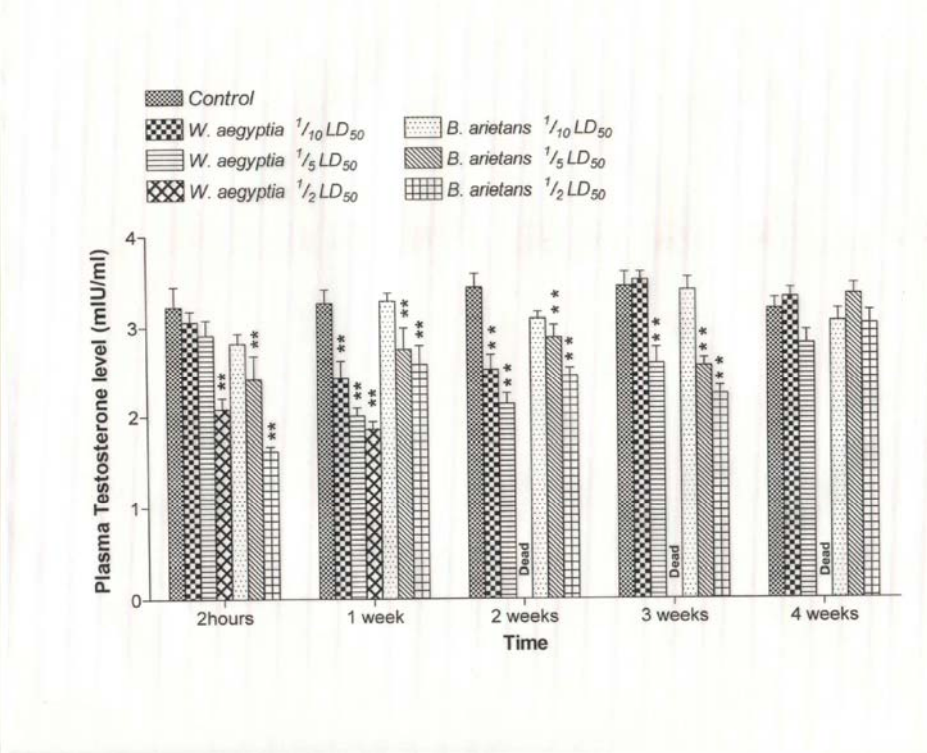

Fig. 1. Effect of *W. aegyptia* and *B. arietans* venoms on plasma testosterone level (mlU/ml) of male rats. Each value is the mean±S.E., n=6.
****: Significant difference p<0.01compared to control.**

LH levels

Figure 2 demonstrated a significant decrease ($p<0.01$) of LH level in the animals treated with *W. aegyptia* venom after 1 week at different doses. Moreover, similar results were recorded in the rats given $\frac{1}{5}$ LD_{50} of the venom after 2 weeks. The hormone level did not change significantly after 3 and 4 weeks with either $\frac{1}{10}$ or $\frac{1}{5}$ LD_{50}.

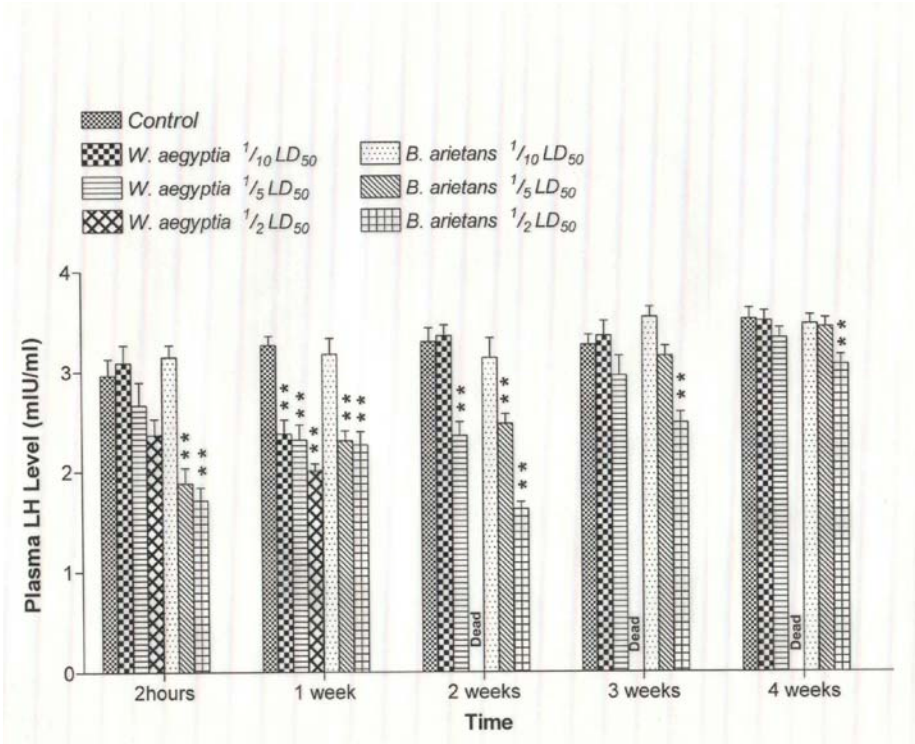

Fig. 2. Effect of *W. aegyptia* and *B. arietans* venoms on plasma LH level (mlU/ml) of male rats.
Each value is the mean±S.E., n=6.
****: Significant difference p<0.01 compared to control.**

A significant decrease ($p<0.01$) in LH level was observed in the rats injected with *B. artietans* venom at $\frac{1}{5}$ LD_{50} and $\frac{1}{2}$ LD_{50} after 2 hours, 1 week and 2 weeks. The significantly decreased ($p<0.01$) is continued in the rats received $\frac{1}{2}$ LD_{50} until the last interval. The hormone was not influenced by $\frac{1}{10}$ LD_{50} of *B. arietans* venom 3 and 4 weeks following the injection.

FSH levels

FSH concentration (Fig. 3) lowered significantly in the rats received $\frac{1}{10}$ LD_{50} of *W. aegyptia* venom after 1 week only, in rats given $\frac{1}{5}$ LD_{50} after 2 hours, 1 week and 2 weeks and in rats injected with $\frac{1}{2}$ LD_{50} after 2 hours and 1 week.

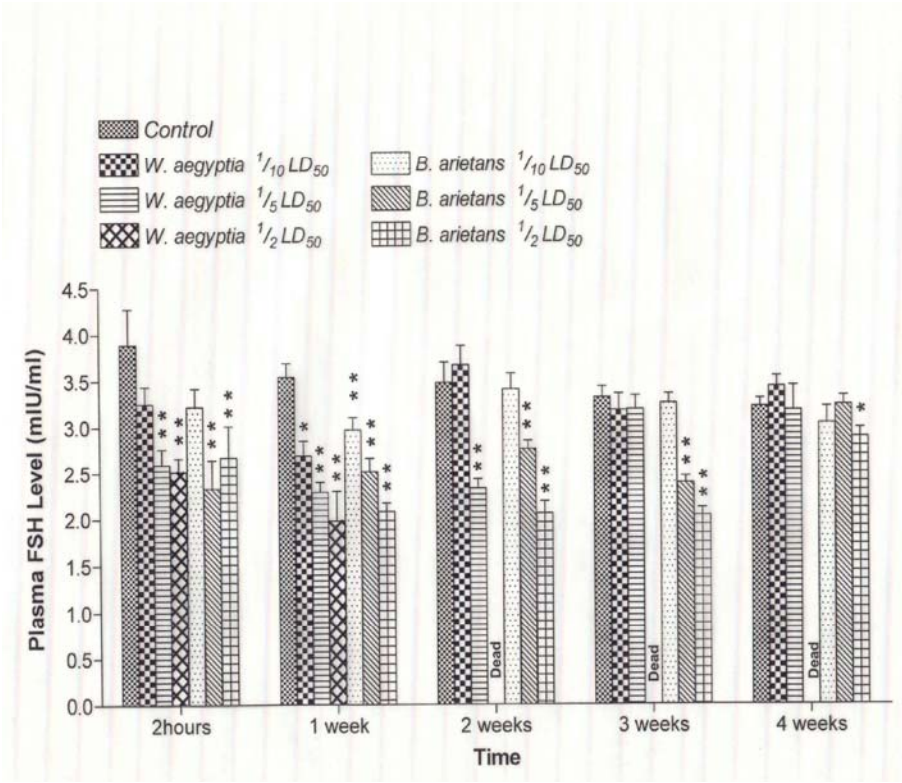

Fig. 3. Effect of *W. aegyptia* and *B. arietans* venoms on plasma FSH level (mlU/ml) of male rats. Each value is the mean±S.E., n=6.
***, **: Significant difference p<0.05, p<0.01 compared to control.**

A highly significant ($p<0.01$) decrease in the hormone level was observed in the rats injected with $\frac{1}{2}$ LD_{50} of *B. arietans* venom at all time intervals. Similar results were recorded to be significant ($p<0.01$) in the case of $\frac{1}{5}$ LD_{50} except the last interval where the decrease was not significant. Moreover, the hormone decreased significantly in the rats given $\frac{1}{10}$ LD_{50} of *B. arietans* venom after 1 week only.

Sperm motility (%)

The treatment of rats with $\frac{1}{5}$ of LD_{50} of *W. aegyptia* venom resulted in a significant ($p<0.01$) decrease of sperm motility at all time intervals (Fig. 4). Similar results were recorded in the case of $\frac{1}{10}$ of LD_{50} after 2 hours, 1 week and 2 weeks. Moreover, the motility also decreased significantly ($p<0.01$) in rats given $\frac{1}{2}$ of LD_{50} 2 hours and 1 week post-injection. The rats injected with $\frac{1}{2}$ of LD_{50} of *B. arietans* venom exhibited a significant decline in sperm motility at all time intervals except the last

interval. Spermatozoal motility was lowered significantly ($p<0.01$) after 2 hours and 1 week only in the rats received $\frac{1}{5}$ of LD_{50} of *B. arietans* venom, while it was not changed significantly in rats given $\frac{1}{10}$ of LD_{50}.

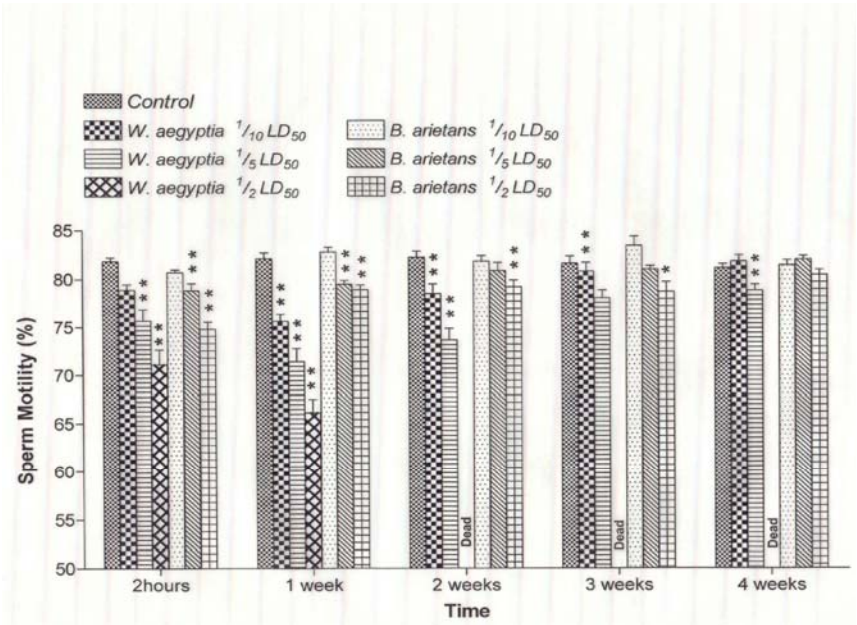

Fig. 4. Effect of *W. aegyptia* and *B. arietans* venoms on sperm motility (%) of male rats.
Each value is the mean±S.E., n=6.
***, **: Significant difference $p<0.05$, $p<0.01$ compared to control.**

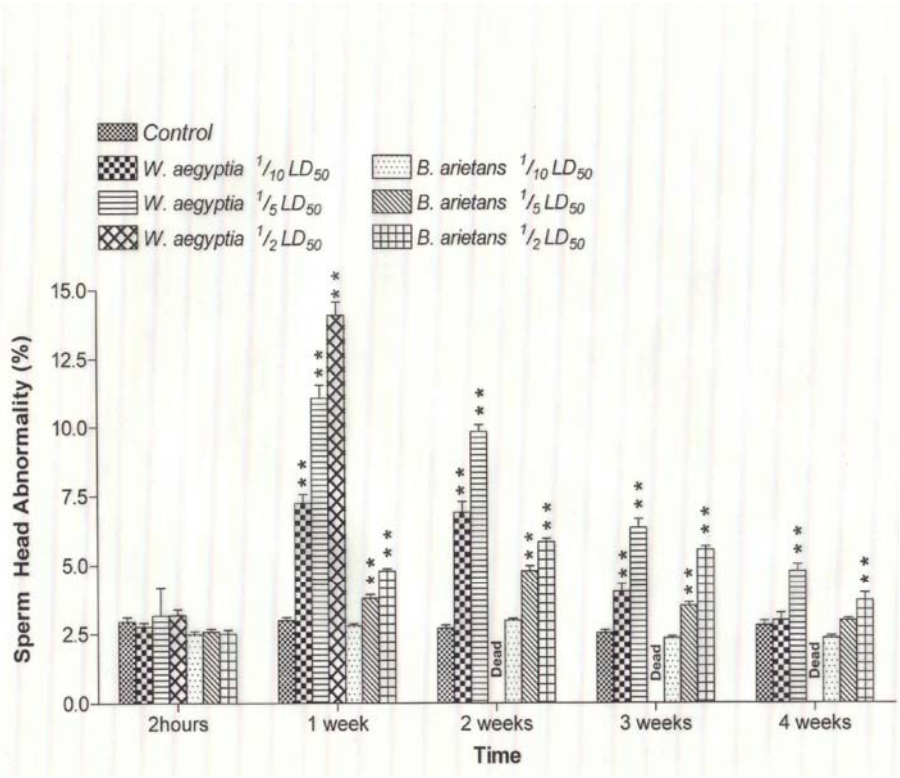

Fig. 5. Effect of *W. aegyptia* and *B. arietans* venoms on sperm head abnormality (%) of male rats. Each value is the mean±S.E., n=6.
****: Significant difference p<0.01 compared to control.**

Sperm abnormality (%)

The animals treated with $\frac{1}{10}$ of LD_{50} or $\frac{1}{5}$ of LD_{50} of *W. aegyptia* venom showed a significant rise ($p<0.01$) in head abnormality after 1, 2 and 3 weeks (Fig. 5). The rise in the head abnormality is continued at 4 weeks in the rats given $\frac{1}{5}$ of LD_{50} of *W. aegyptia* venom. In the rats injected with $\frac{1}{2}$ of LD_{50} of *B. arietans* venom, the head abnormality elevated significantly ($p<0.01$) in all time intervals except after 2 hours. Similar results were observed after 1, 2 and 3 weeks in the rats given $\frac{1}{5}$ of LD_{50} of *B. arietans*. The venom of *B. arietans* at the dose level of $\frac{1}{10}$ of LD_{50} has no significant effect on sperm head abnormality.

The tail abnormality (%) of rats treated with *W. aegyptia* venom showed a significant increase ($p<0.01$) at the three doses at 1, 2 and 3 weeks intervals, while it has normal values in the rats treated with *B. arietans* venom (Fig. 6).

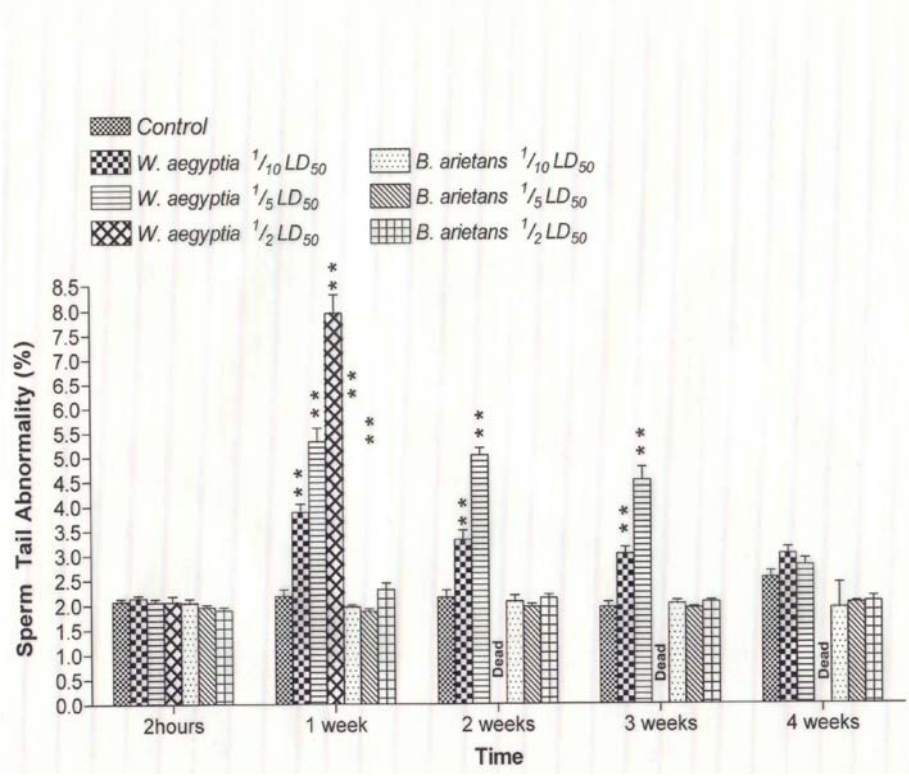

Fig. 6. Effect of *W. aegyptia* and *B. arietans* venoms on sperm tail abnormality (%) of male rats.
Each value is the mean±S.E., n=6.
****: Significant difference p<0.01 compared to control.**

The percentage of total sperm abnormality (Fig. 7) in rats injected with $\frac{1}{5}$ of LD_{50} of *W. aegyptia* venom and $\frac{1}{2}$ of LD_{50} of *B. arietans* venom elevated significantly ($p<0.01$) at all time intervals except after 2 hours. Moreover, the total spermatozoal abnormality of animals given $\frac{1}{10}$ of LD_{50} of *W. aegyptia* venom and $\frac{1}{5}$ of LD_{50} of *B. areitans* venom increased significantly after 1, 2 and 3 weeks. The total sperm abnormality (%) increased significantly ($p<0.01$) in the rats received $\frac{1}{2}$ of LD_{50} of *W. aegyptia* venom after 1 week of injection.

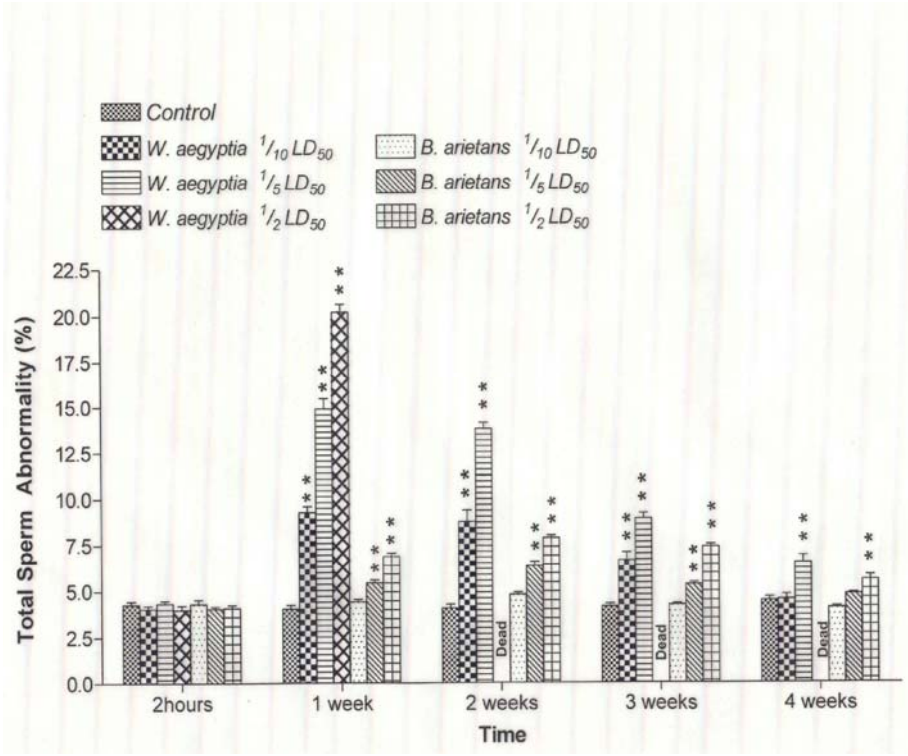

Fig. 7. Effect of *W. aegyptia* and *B. arietans* venoms on sperm total abnormalities (%) of male rats. Each value is the mean±S.E., n=6.
****: Significant difference p<0.01 compared to control.**

Sperm count per epididymis

The sperm count decreased significantly ($p<0.01$) in the rats injected with $\frac{1}{5}$ of LD_{50} of *W. aegyptia* venom after 3 and 4 weeks (Fig. 8), while it did not change significantly in the rats treated with *B. arietans* venom at the same dose. No significant changes observed in the rats treated with $\frac{1}{10}$ of LD_{50} of the two tested venoms. Moreover, a significant decrease ($p<0.01$) in the sperm count was recorded in the rats given $\frac{1}{2}$ of LD_{50} of *B. arietans* venom after 3 weeks only.

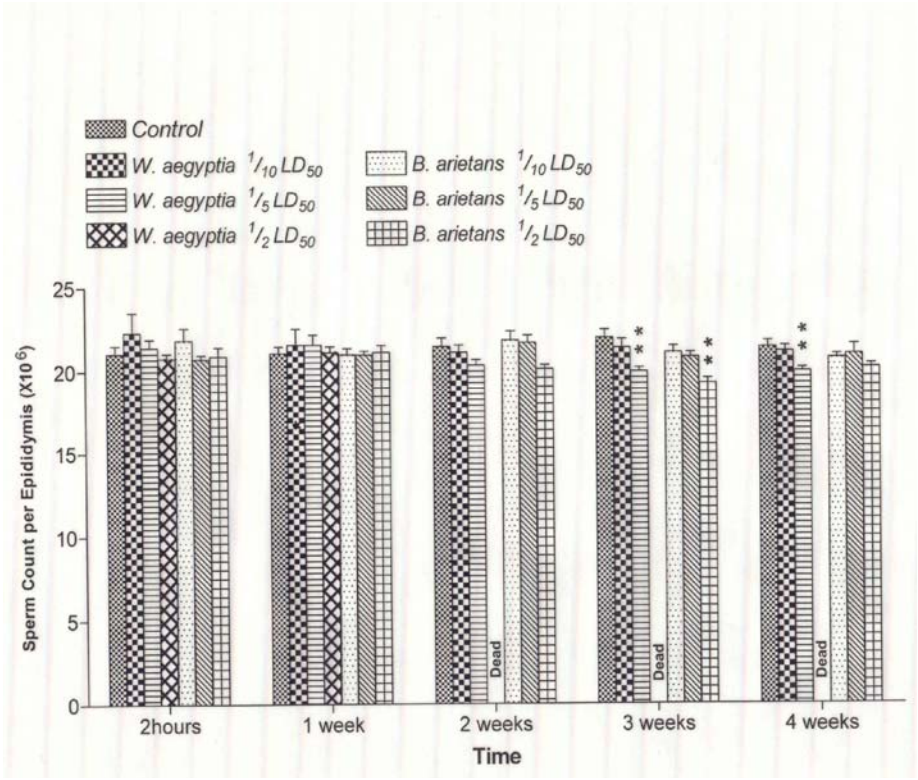

Fig. 8. Effect of *W. aegyptia* and *B. arietans* venoms on sperm count per epididymis (X10^6) of male rats. Each value is the mean±S.E., n=6.
****: Significant difference p<0.01 compared to control.**

Discussion

The present experiments indicated a decrease in testosterone, LH and FSH levels in the rats injected with *W. aegyptia* and *B. arietans* venoms and this decrease is a dose dependent. The decrease in the testosterone level could be explained by the continual increase in the vascular permeability of the testis and the degenerative changes that would occur in the testicular tissues in response to the prolonged effect of the toxins that would consequently result in a significant drop in testicular blood flow and testosterone [13]. Several reports of chronic pituitary alteration that resulted in low serum testosterone concentration were documented in human subjects following bites by Russel's vipers 2 weeks after enveomotion [14, 15]. The effects of venoms from cobra and vipers in Saudi Arabia on testosterone levels were stimulatory with acute treatment or inhibitory with chronic treatment depending on the vascular blood flow and testicular degeneration [16]. However, Waites and Setchell [17] studied the toxicity of cadmium salts to the testis and obtained increased blood flow and increased permeability of the blood testis barrier (BTB).

Reduction in testis weights and testosterone concentration were reported following chronic treatment with aflatoxins fed to male chicken [18], while testicular atrophy with morphological and histological changes were observed in young broilers fed with triorthocresyl phosphate neurotoxin [19]. The injection of the present venoms may produce chronic effects and hence the testicular blood flow was gradually decreased resulting in a significant drop in testosterone concentration. A significant correlation was reported between the testicular blood flow and testosterone concentration [20].

Spermatocytes arrested with low sperm volume were observed in mice injected with scorpion venom [8]. In the present study, sperm motility decreased in the rats envenomed with *W. aegyptia* or *B. arietans* venom.

It was reported that the injection of venoms to animals can increase the levels of lipid peroxidation [21] and hydrogen peroxide [22]. It is known that both lipid peroxidation and hydrogen peroxide are very toxic to the cells; hence they may adversely affect the sperm motility. The observed decrease in the sperm motility can be attributed to the low testosterone level, since the hormone control the sperm motility. Alhazza and Bashandy [23] reported that the treatment of male rats with *W. aegyptia* and *B. arietans* venoms led to a decrease in RBCs count, packed cell volume and hemoglobin concentration. As a result, the concentration of oxygen reached the organs may decrease leading to a decrease in sperm motility, since the sperm contains mitochondria that needs oxygen to produce energy used for sperm motility.

The percentage of sperm abnormalities increased in the present work due to the injection of rats with *W. aegyptia* or *B. arietans* venom. Moreover, the sperm count per epidiodymis decreased in rats given *W. aegyptia* venom at the dose level of $\frac{1}{5}$ LD_{50} after 3 and 4 weeks, and in rats injected with $\frac{1}{2}$ LD_{50} of *B. arietans* venom at 3 weeks. The decrease in the sperm count is likely due to a decrease of testosterone, LH and FSH, since these hormones are essential for normal spermatogenesis.

In conclusion, the present results indicate that *W. aegyptia* and *B. arietans* venoms have adverse effects on pituitary-gonadal axis and spermatogenesis.

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تأثير سم الصلّ (*Walterinnesia aegyptia*) والأفعى النوامة (*Bitis arietans*) على الهرمونات المنسلية والجنسية، والحيوانات المنوية في ذكور الجرذان

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(قدم للنشر في ١٤٢٧/١١/٢٠هـ؛ وقبل للنشر في ١٤٢٨/١/١٩هـ)

ملخص البحث. تشكل عضّات الأفاعي خطرا صحيا عاما في المملكة العربية السعودية. تم في هذه الدراسة الكشف عن تأثير سمّ نوعين (الصلّ والأفعى النوّامة) من الأفاعي التي تعيش في المملكة، على مستويات الهرمون اللوتيني والهرمون المحفز للجريبات وهرمون التستستيرون في الدم، وعلى خصائص الحيوانات المنوية في ذكور الجرذان خلال أوقات مختلفة بعد الحقن.

لم يؤثر سمّ الصلّ على مستوى الهرمون اللوتيني والتستوستيرون عند عُشر LD_{50} بعد أسبوع من الحقن، بينما انخفض مستوى الهرمون المحفز للجريبات لكلا السمّين عند نفس الجرعة والفترة. جميع الهرمونات المدروسة انخفضت عند خُمس ونصف LD_{50} لكلا السمّين.

بدا تأثير سمّ الصلّ واضحًا على خصائص الحيوانات المنوية مقارنة بسم النوّامة عند عشر LD_{50}، بينما ظهر أثر السمّ جليًّا عند خُمس ونصف LD_{50} لكلا السمّين. انخفض عدد الحيوانات المنوية عند خُمس LD_{50} لسمّ الصلّ بعد ثلاثة وأربعة أسابيع من الحقن، بينما لم يحدث الأثر نفسه عند عُشر LD_{50} لنفس الفترات.

والخلاصة هي أن تأثير سمّ الصلّ والنوّامة على الهرمونات المنسليّة والجنسيّة، وعلى الحيوانات المنوية يعتمد على الجرعة والفترة.

الكلمات المفتاحية: السعودية، الصل، النوامة، سم الثعابين، حيوانات منوية، هرمون.