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EFFECT OF DIAZEPAM ON THE TESTICULAR HISTOLOGY AND GENE EXPRESSION IN RATS

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ABSTRACT

Steroid biosynthesis is initiated with transportation of cholesterol along the steroidogenic acute regulatory protein (StAR) into the mitochondria. It has been reported that Ca^{2+} channel blockers suppress the biosynthesis of androgens. Diazepam is one of the most common benzodiazepines used for anxiety. Recent studies found that diazepam acts as Ca channels antagonist as it can produce a complete inhibition of voltage-dependent Ca uptake. The purpose of the present study was to investigate whether treatment of male rats with diazepam interferes with steroidogenesis. Fifty male rats were allocated into five groups. Control (D.W.) (n=10) and test groups that received (2, 5 and 10 mg/kg/day) of diazepam by oral gavage, each group (n=10) and sulfasalazine (500mg/kg) for 8 weeks. Animals were kept in standard conditions. At the end of the treatment; animals were sacrificed and the epididymis was removed. Sperms were collected and the sperm count, motility, viability and morphology were determined. The testis were also removed and the effect of diazepam on the steroidogenic acute regulatory protein (StAR) mRNA expression was assessed by using reverse transcription (Reverse-transcriprase polymerase chain reaction) analysis. Testicular histological analysis was also achieved. The results of the present study showed a significant decrease in sperm count, motility and viability with an increase in sperms abnormalities. Also showed that diazepam significantly inhibits the (StAR) mRNA expression. Histological analyses showed dose dependent anomalies of the testis. From the data; it can be concluded that diazepam, in a dose dependent pattern, was effective in attenuating steroidogenesis production through an inhibitory effects on StAR protein gene expression in rats.

KEYWORDS: steroidogenic, Ca channels antagonist, Ca uptake and StAR.

INTRODUCTION

Calcium ion is implicated in diverse cellular functions in both germ cells and somatic cells in the testis, particularly, mediating the responses to endocrine hormones and local regulators in genital tracts.[1,2] A common belief is that the Ca^{2+} influx and efflux should be tightly regulated to maintain the intracellular Ca^{2+} homeostasis, and an alteration in the Ca^{2+} transport across the cell membrane could result in a drastic impact on spermatogenesis and steroidogenesis.[3, 4] Leydig cell production of testicular androgens is tightly controlled by endocrine interactions among the pituitary gland and the testis, as well as through the paracrine and autocrine regulation within the testis.[5, 6, 7] Leydig cells secrete testosterone responsible for the onset of both spermatogenesis and male sexual development. Endocrine control of Leydig cell steroidogenic activity by luteinizing hormone (LH), follicle-releasing hormone (FSH) or human chorionic gonadotropin (hCG) has been exerted through their Ca^{2+}- mediated signaling pathway.[8, 9, 10]

Recent studies suggest that Ca^{2+} affects the transfer of the substrate cholesterol to the inner mitochondrial membrane, the rate-limiting step in steroidogenesis.[11, 12] This was confirmed by a study reporting Ca^{2+} induced increase in steroidogenic acute regulatory (StAR) protein[13], which is critical for the cholesterol transfer to the inner mitochondrial membrane to initiate steroidogenesis.[14,15,16] Diazepam is a benzodiazepine that is widely used as an anxiolytic, anticonvulsant, hypnotic, sedative & skeletal muscle relaxant. Recent studies found that benzodiazepines can produce a complete inhibition of voltage-dependent $Ca2^{+}$ uptake. Also, they indicate that benzodiazepines are acting as $Ca2^{+}$ channels antagonists.[17]

MATERIALS AND METHODS

Animals

Sprague-Dawly male rats weighing 250-300 gm and 8 weeks old were obtained from the animal house of the College of Pharmacy- University of Baghdad. The animals were maintained on normal conditions of temperature, humidity and light/dark cycles. They were fed standard rodent pellet and they have free access to water.

The study design

Fifty male rats were used in the present study, the study groups were divided into 5 groups: First group (control): 10 rats were administered distilled water for 8 weeks by oral gavage. Second group: 10 rats were used for the study of the infertility activity of diazepam in which (2mg/kg BW) of diazepam was given for 8 weeks by oral gavage.

Third group: 10 rats were used for the study of the possible infertility activity of diazepam in rat model. In this group (5mg/kg BW) dose of diazepam were used for 8 weeks by oral gavage.

Fourth group: 10 rats were used for the study of the possible infertility activity of (10mg/kg BW) diazepam was used for 8 weeks by oral gavage.

Fifth group: 10 rats were given a dose of (500mg/kg BW) of sulfasalazine for 8 weeks by oral gavage as a positive control (in this group, sulfasalazine represents standard infertility agent.

Epididymal tail suspension preparation

At the end of treatment; the cauda epididymis was quickly removed into a petridish that contains 10 ml of warm normal saline at 37°C and it was cut longitudinally with a pair of fine pointed scissors and compressed with forceps. The sperms were released by mincing the cauda epididymis into pieces (at least 200 cuts) to perform the following microscopical examination on sperm characters.[18]

Determination of sperm concentration

Sperm count was determined using the haemocytometer under light microscope. A cover slip was placed on the haemocytometer before a drop of the epididymal sperm solution was loaded under the cover slip. Sperm count was done by counting 5 RBC small squares.

Sperm count was determined using the following formula:

Sperm count= total no. of sperms in 5 squares ×50,000×100 (cells/ml).[19]

Determination of sperm motility

Sperm motility was assessed by placing a drop of the sperm suspension over a clean dry slide and covered with a cover slip and then the slide was placed under light microscope. The data were tabulated in the form of percentage using the formula:

Percentage of motile sperms =no. of motile sperms ×100%/total no. of sperms (motile and immotile).[20]

Determination of sperm viability

In this analysis; a drop from the sperm suspension used before was mixed with one drop of eosin and then after 30 seconds, a drop of nigrosin was added and mixed. Then a smear was made. After awhile, the smear was air-dried and observed under light microscope. The dead

sperms showed pink color of the head while the viable sperm showed colorless or whitish head based on the degree of membrane permeability, then the data were tabulated in the form of percentage using the following formula.

Percentage of viable sperms =no. of viable sperms×100%/ total no. of dead and viable sperms.[18]

Determination of sperms abnormalities

In this analysis; the same sperm smears made for sperm viability were observed under light microscope. The smears were examined for abnormal morphology of the head, neck and tail. Then data were tabulated using the following formula.

Percentage of abnormal sperms =no. of abnormal sperms×100%/ total no. of normal and abnormal sperms.[21]

Histological evaluation of tissues

After the end of treatment; the testes were excised and cleared off the attached fat and connective tissues. Histological sections were prepared according to Luna (1968) methods for histological evaluation.[22]

C DNA synthesis and purification of the StAR cDNA using reverse transcriptase – polymerase chain reaction (RT- qPCR).

Extraction of RNA from tissue

Total RNA was extracted from fresh tissue (testes) using the Geneaid total RNA mini kit which was designed specifically for purifying total RNA from a variety of fresh and paraffin – embedded tissues.[23]

Determination of RNA yield and purity

The most common and easiest technique to determine RNA yield and purity is absorbance using nanodrop instrument which is highly sensitive and directly provides the concentration of RNA in ng/ml. the measurement depended on the ratios of absorbance at 260 and 280 nm, i.e A260/A 280 ratio.

Conversion of RNA into cDNA using RT-PCR

The RNA extracted previously from tissues is converted into cDNA using AccuPower® Rocket Script ™ RT premix kit from Bioneer. AccuPower® Rocket Script ™ RT premix is a

ready to use lyophilized master mix containing all components for first strand cDNA synthesis from a purified total RNA template.

The reaction is performed under the following conditions:

Table (1): Conditions for conversion of RNA into cDNA.

Step	Temperature	Time
cDNA synthesis	42-70°C	1 hr
Heat inactivation	95 °C	5 min

The cDNA formed previously was checked using agarose gel electrophoresis.

The agarose gel electrophoresis was done according to Harisha method.[24] Preparation of the agarose gel was according to Lee method[25] and the ethidium bromide staining was done according to Robinson and Lafleche method.[26]

Agarose gel was visualized in a UV transilluminator and photos were captured.

Real time PCR (qPCR)

The cDNA was amplified using Accupower®Green Star™ qPCR premix kit from Bioneer. Accupower®Green Star™ qPCR premix is a ready to use reagent containing all components for real- time PCR reaction just by the addition of a specific primer and target gene into tubes provides results with high sensitivity and specificity. There are three major steps at different temperatures in a PCR which are repeated for 30 or 45 cycles. Double stranded target DNA is heat denaturated (denaturation step), the two primers complementary to the target segment are annealed at low temperature (annealing step), and the annealed primers are then extended at an intermediate temperature (extension step) with a DNA polymerase. As the target copy number doubles each cycle, PCR can thereby amplify DNA fragments up to 10^{8} –fold in a short period.[27]

The PCR products are detected with SYBR Green dye. SYBR Green fluorescence is enormously increased upon binding to double- stranded DNA. During the extension phase, more and more SYBR Green will bind to the PCR product resulting in an increased fluorescence. Consequently, during each subsequent PCR cycle, more fluorescence will be detected.

The PCR setting is shown in table (2):

Table (2) Conditions for real-time PCR.

Step	Condition		Cycle
Pre-denaturation	95 °C	1min	1
Denaturation	95 °C	5 sec	40
Annealing/Extension	55°C	40 sec	
Detection(Scan)			
Melting	55°C	1 sec	1

The oligonucleotide primer sequence used for PCR amplification of StAR gene (Genebank:Access no.BC060970) is shown in table (3).

Table (3) primer sequence for the StAR gene.

Primer	Sequences
Forward primer	LP5´-GAC CTT GAA AGG CTC AGG AAG AAC-3´
Reverse primer	RP5´-TAG CTG AAG ATG GAC AGA CTT GC-3´

The oligonucleotide primer sequence used for PCR amplification of ß-actin gene (Genebank:Access no.NM007393) is shown in table (4).

Table (4) primer sequence for the ß-actin gene.

Primer	Sequences
Forward primer	LP5´- ATGCCCACTGCCGCATCCTCTTCC -3´
Reverse primer	RP5´- CACGATGGAGGGGCCGGACTCATC -3´

The data results of qRT –PCR for the target (StAR) and housekeeping gene (ß-actin) were analyzed by the relative quantification gene expression levels (fold change) Livak method that described by.[28]

In which the following formula was applicated:

Relative expression ratio= $2^{-\triangle\triangle Ct}$

Statistical analysis

Analysis of data was carried out using the available statistical package of SPSS-21 (Statistical Packages for Social Sciences version -21). Student t-test was used for testing the significance of difference between two groups and ANOVA between three. Statistical significance was considered whenever the (p value) was equal to or less than 0.05.

RESULTS

Sperm concentration

The data referring to sperm concentration in epididymal suspension of control and treated groups are shown in table (5).

Table (5): Effect of different oral doses of diazepam suspension and sulfasalazine on the sperm characteristics of male rats.

Group	Controls	T1(2mg/kg)	T2(5mg/kg)	T3(10mg/kg)	Sulfasalazine (500mg/kg)
Sperm conc.(million/ml)	644.5±16.29	529.9±6.56 **a	400.2±8.49 **b **d	292.4±9.30 **c**e**f	211±9.19 **g**h**i**j
Motility (%)	91.7±0.86	86.5±1.79 *a	77.9± 1.83 **b **d	64.7± 1.85 **c**e**f	58± 1.38 **g**h**i*j
Viability (%)	84.9± 1.33	81.1± 1.5 Ns	70.8± 1.65 **b **d	67.7± 1.01 **c **e Ns	65.8± 1.16 **g**h*i Ns
Abnormality (%)	5.13±0.363	9.88±0.436 **a	28.24±0.923 **b **d	30.74±1.42 **c **e *f	31.62±0.648 **g**h*i Ns

Data are expressed as mean (±SE); n=10 rats/group; a: t-test between control and T1, b: t-test between control and T2, c: t-test between control and T3, d: t-test between T1 and T2, e: t-test between T1 and T3, f: t-test between T2 and T3, g: t-test between control and sulfasalazine, h: t-test between T1 and sulfasalazine, i: t-test between T2 and sulfasalazine, j:t-test between T3 and sulfasalazine, *:significant ($p<0.05$) difference, **:highly significant ($p<0.001$) difference, NS: no significant difference.

Table (5) showed that the sperm concentration, motility and viability decreased highly significantly ($p< 0.001$) in the T1 (2mg/kg), T2 (5mg/kg), T3 (10mg/kg) groups and in the sulfasalazine group compared to the control group. While the percentage of sperms abnormalities increased also highly significantly in all treatment doses and the sulfasalazine group than in the control group.

The histological changes in testicular tissues

The microscopic study of rat´s testes treated with diazepam suspension for 8 weeks revealed variable degrees of alteration according to the dose of treatment compared to the control group. Many histological changes in semniferous tubules, spermatogenesis and number of sperms in the lumen of semniferous tubules are occurred.

Figure (1) show sections of the control rat testis with normal structure appearance of semniferous tubules and full maturation of spermatogonia cells and sperms present inside the lumen (normal spermatogenesis).

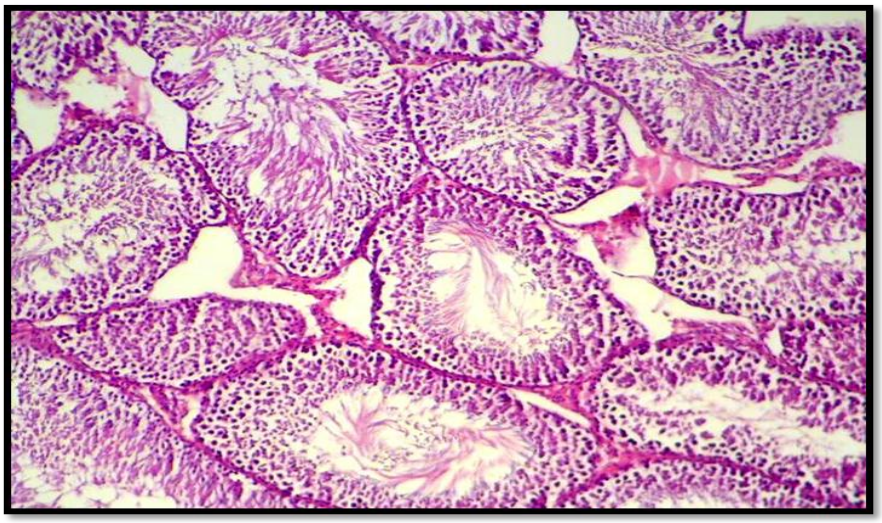
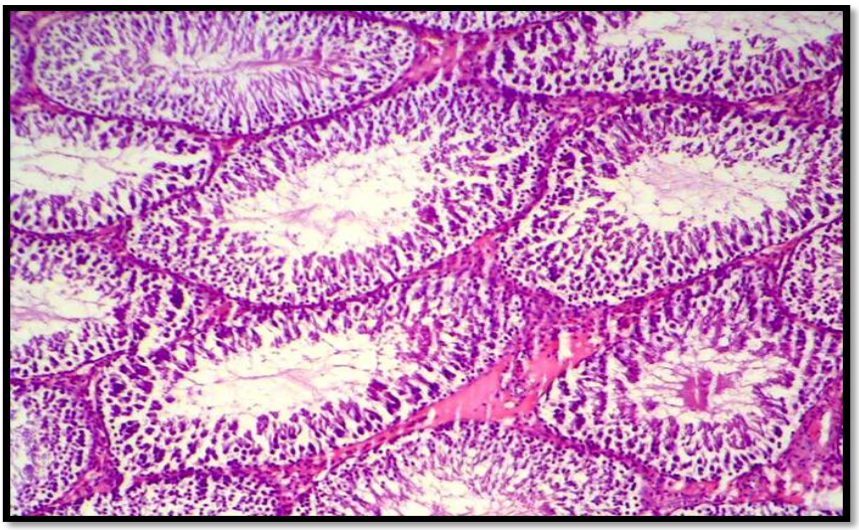


Figure (1) Cross section of normal rat´s testes (control group). (H& E ×20).

Figure (2) show sections of rat´s testis treated with 2mg/kg of diazepam suspension in which certain semniferous tubules are empty from the production of sperms.



Figurev (2) Cross section of rat´s testes treated with 2 mg/Kg of diazepam suspension. (H&E ×20), black arrows showing few sperms.

Figure (3) show sections of rat´s testis treated with 5mg/kg of diazepam suspension in which certain tubules show damage of maturity of sperm production cells (primary spermatocytes, secondary spermatocytes and spermatids).

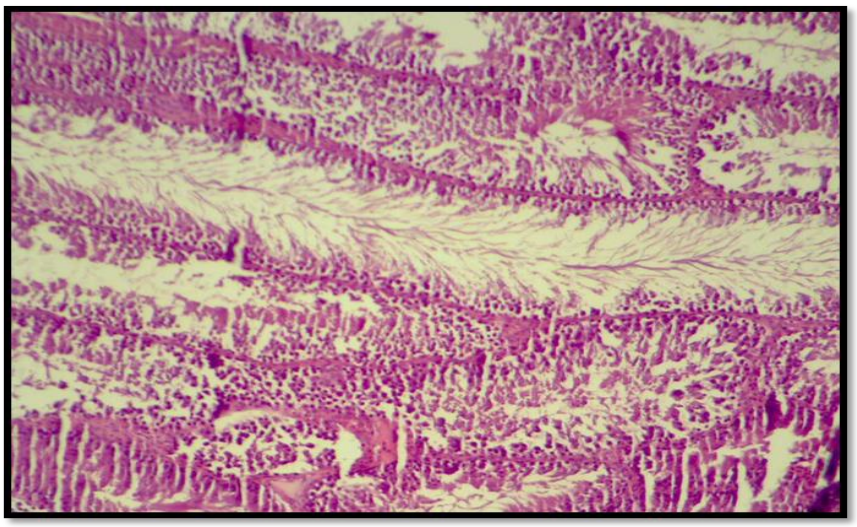


Figure (3) Cross section of rat´s testis treated with 5 mg/Kg of diazepam suspension. (H&E ×200), black arrows showing no sperms.

Figure (4) show sections of rat´s testis treated with 10mg/kg of diazepam suspension in which there is some severe necrosis of products with certain necrosis of spermatogonic cells, other show no products of sperm.

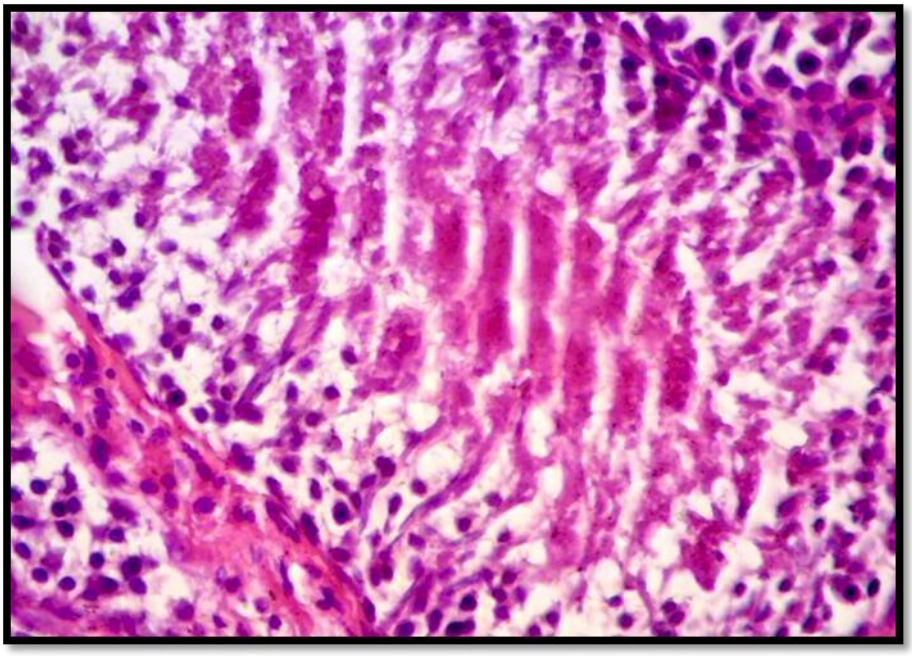


Figure (4) Cross section of rat´s testis treated with 10 mg/Kg of diazepam suspension. (H&E ×40), black arrows showing spermatogonic cell necrosis.

Figure (5) show sections of rat´s testis treated with sulfasalazine showing immaturation of spermatogonia cells with few sperms inside the lumen.

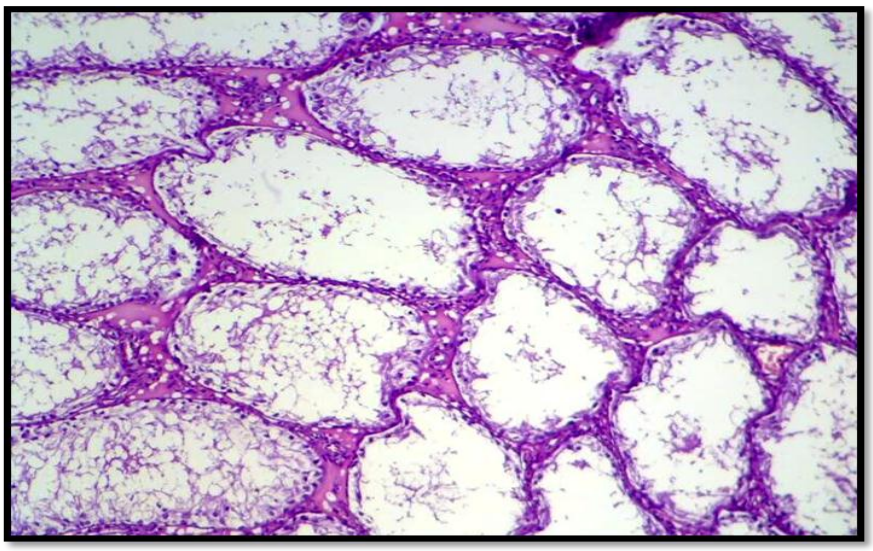


Figure (5): Cross section of rat´s testis treated with sulfasalazine. (H&E×20).

Quantitative Reverse transcriptase Real – time PCR

Quantitative Reverse transcriptase Real – time PCR (RT-q PCR) was performed for measurement of relative quantification (gene expression analysis) for StAR gene expression level normalized by housekeeping gene expression (ß-actin). RT-q PCR quantification method in real-time PCR system was dependent on the values threshold cycle numbers (CT) of amplification plot of target gene and housekeeping gene.

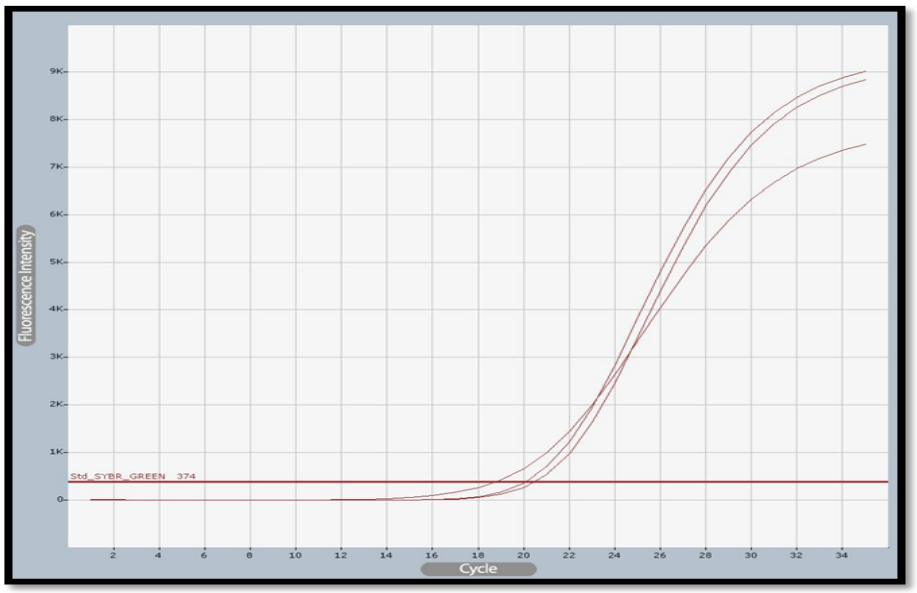


Figure (6) amplification plot for StAR gene in the control.

Figure (7) amplification plot for ß-actin gene in the control.

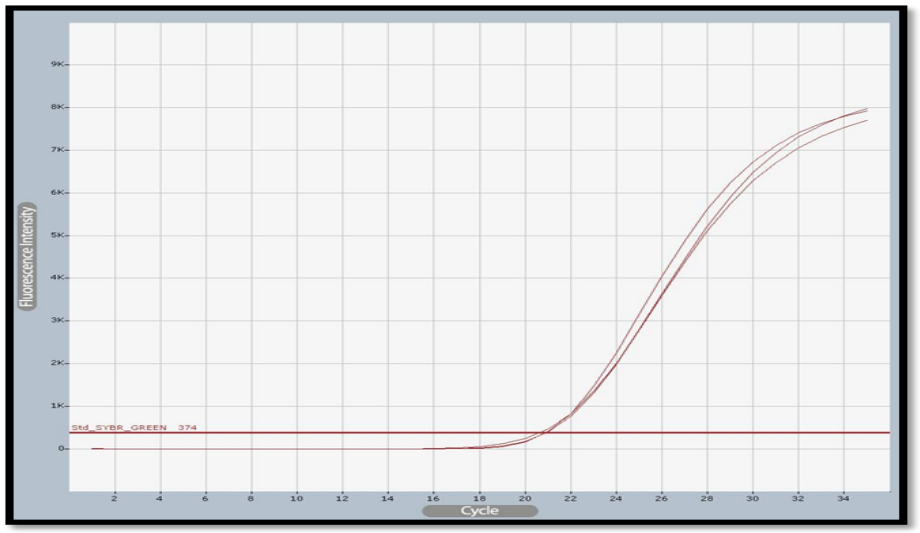


Figure (8) amplification plot for StAR gene in 2mg/kg dose of diazepam.

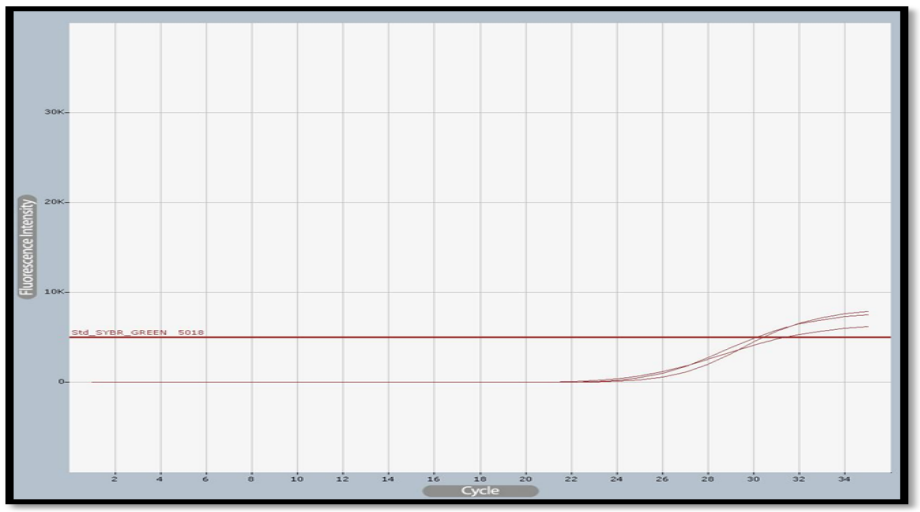


Figure (9) amplification plot for ß-actin gene in 2mg/kg dose of diazepam.

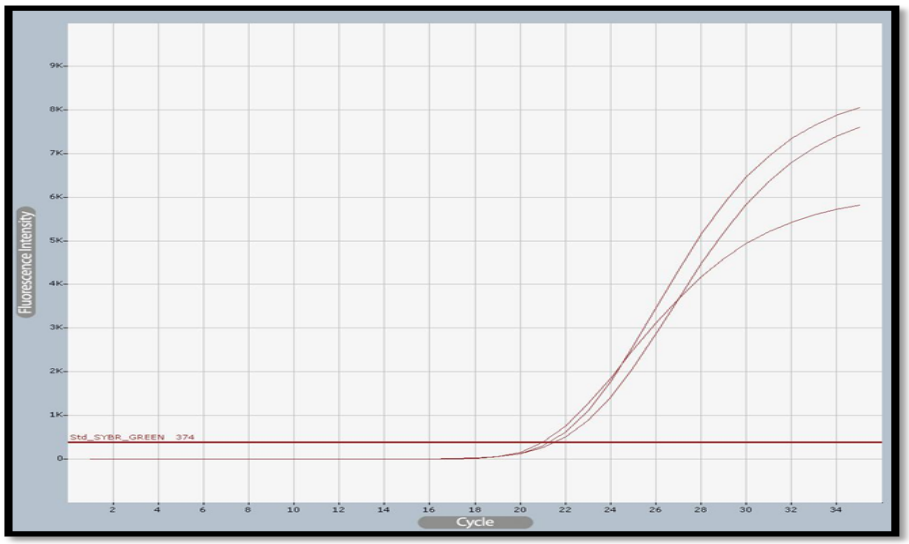


Figure (10) amplification plot for StAR gene in 5mg/kg dose of diazepam.

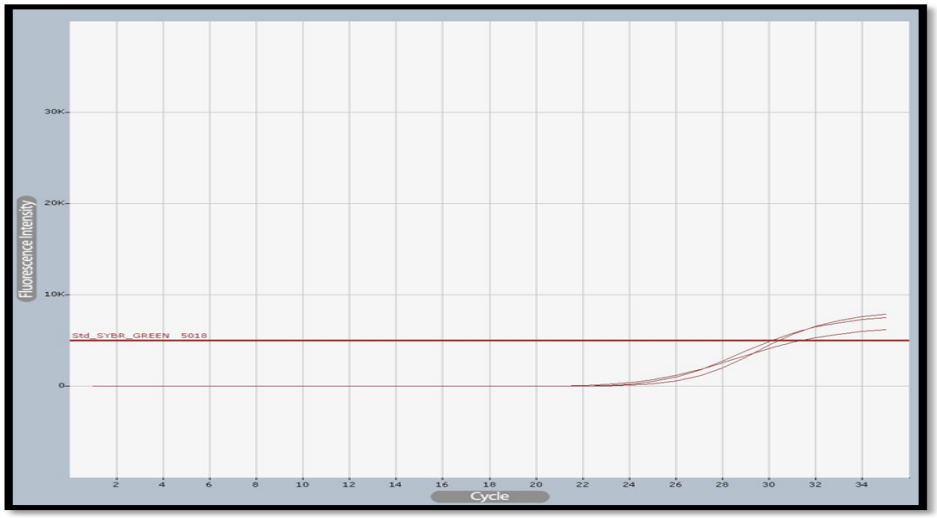


Figure (11) amplification plot for ß-actin gene in 5mg/kg dose of diazepam.

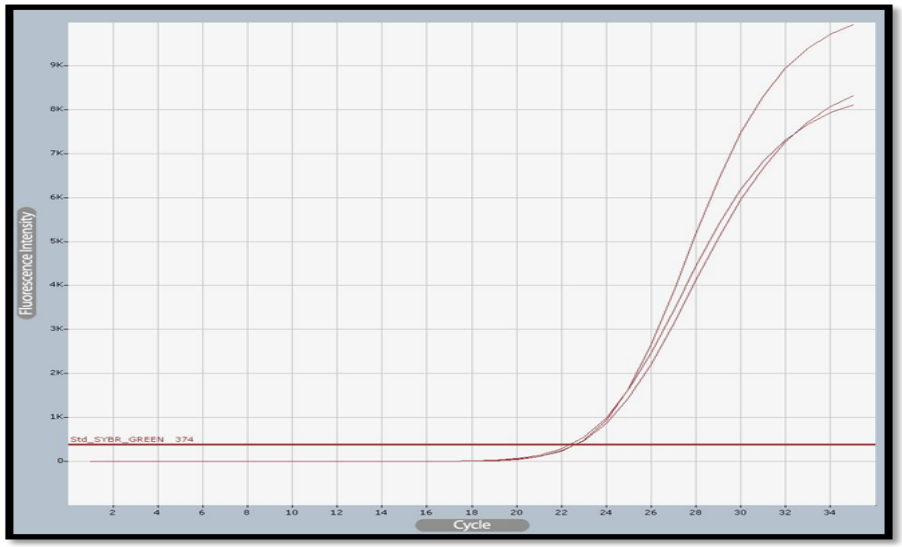


Figure (12) amplification plot for StAR gene in 10mg/kg dose of diazepam.

Figure (13) amplification plot for ß-actin gene in 10mg/kg dose of diazepam.

Figure (14) amplification plot for StAR gene in sulfasalazine treatment.

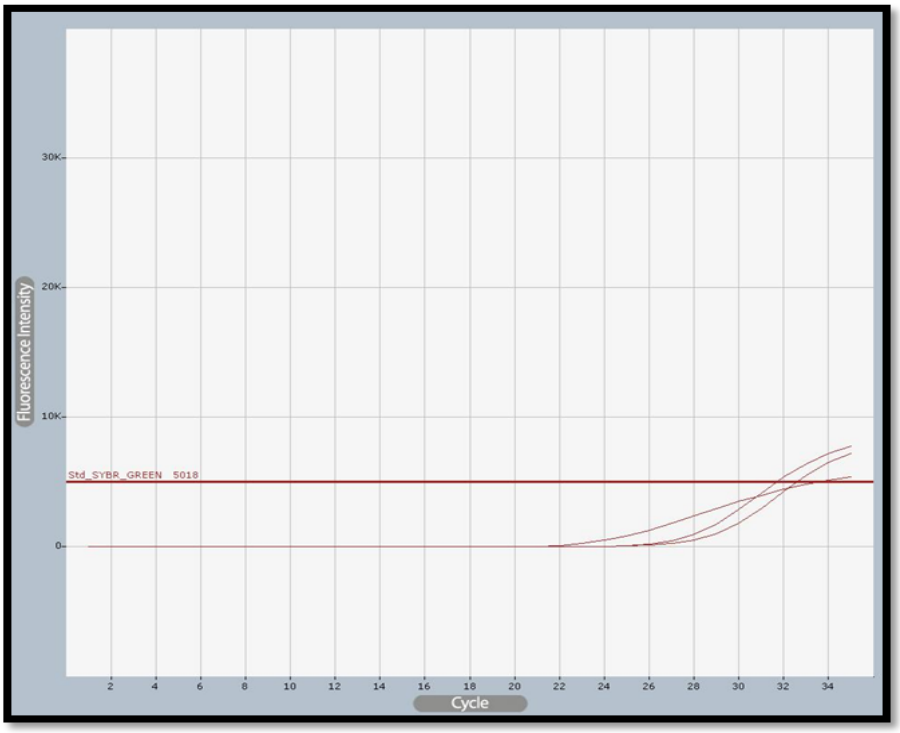


Figure (15) amplification plot for ß-actin gene in sulfasalazine treatment.

Table (6) relative gene expression between groups using RT-qPCR.

Group	Control	T1(2mg/kg)	T2(5mg/kg)	T3(10mg/kg)	sulfasalazine
Relative StAR gene expression	1	1.305±0.312	0.888±0.069 **a NS	0.66±0.038 **b*d Ns	0.45±0.086 **c*e*f NS

Data are expressed as mean (±SE); n=3 rats/group; a: t-test between T1 and T2, b: t-test between T1 and T3, c: t-test between T1 and sulfasalazine, d: t-test between T2 and T3, e: t-test between T2 and sulfasalazine, f: t-test between control and sulfasalazine, *:significant ($p<0.05$) difference, **:highly significant ($p<0.01$) difference, NS: no significant difference.

Relative gene expression

The relative expression of the target gene (StAR) in male rats testes was calculated by using Livak method ($2^{-\triangle\triangle CT}$) that is dependent on normalization of RT-qPCR(CT values)of the target gene with housekeeping gene (ß-actin) as reference gene in control and treatment groups. Our results of the relative gene expression in StAR gene showed highly significant ($p<0.001$) difference in fold change of the gene expression levels between control and treatment groups. It showed that the relative gene expression of the StAR gene of the T2 (5mg/kg), T3 (10 mg/kg) and the sulfasalazine group; the results were (0.888±0.069; 0.66±0.038 and 0.45±0.086) respectively; were highly significantly ($p<0.001$) decreased than the T1 (2mg/kg); (1.305±0.312) group. Also, the relative StAR gene expression of the T3

(10mg/kg); (0.66±0.038) and sulfasalazine (0.45±0.086) were significantly ($p<0.05$) decreased than the T2 (5mg/kg); (0.888±0.069) group. And the relative gene expression of the sulfasalazine (0.45±0.086) group was significantly ($p<0.05$) decreased than the control group which is equal to 1 fold change of gene expression levels. while there was no significant difference between both the T2 (5mg/kg); (0.888±0.069) and T3 (10mg/kg); (0.66±0.038) groups compared to the control (1) group and no significant difference between the T3(10mg/kg); (0.66±0.038) and the sulfasalazine (0.45±0.086)groups.

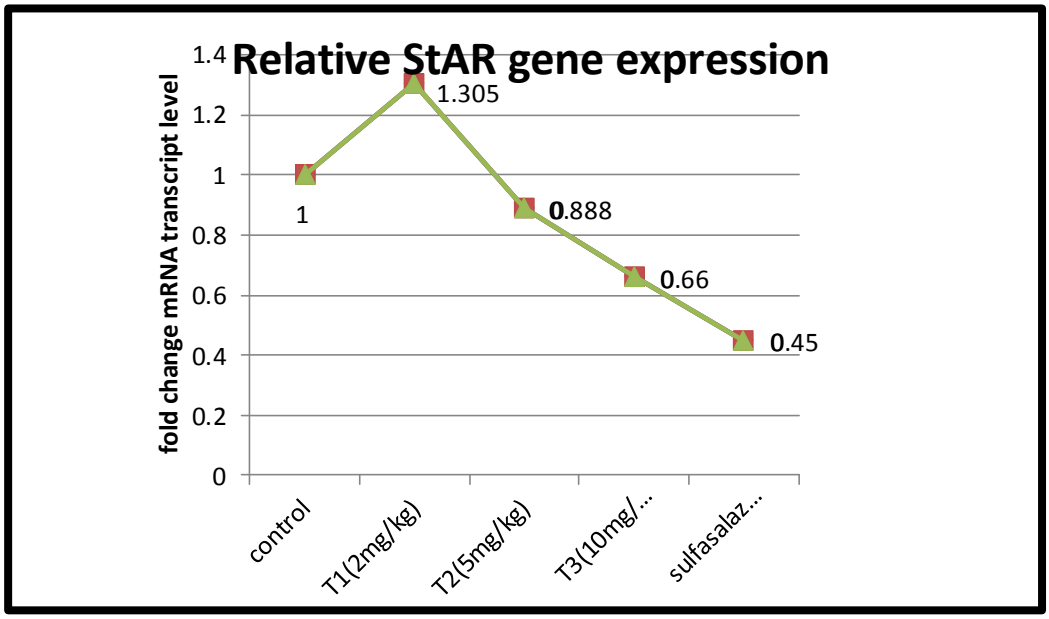


Figure (16) gene expression of StAR by 2 $^{-\Delta\Delta CT}$ Livak method.

DISCUSSION

In table (5); the sperm count was observed to have reduced significantly, which is an indication that diazepam suspension had inhibited the spermatogenesis process.[29] Spermatogenesis is influenced by the hypothalamic- pituitary- testicular axis relating gonadotropin releasing hormone, LH, FSH and androgens. Thus, the effects evoked by diazepam on sperm concentration might be strongly linked with status of LH and FSH hormones which are also reduced and greatly affect sertoli cells functions in the testes specially sperm production.[30] This implies that the decrease in sperm count caused by the drug in the treated rats was as a result of a decrease in plasma level of testosterone, because this hormone has been reported to be important in initiation and maintenance of spermatogenesis.[31, 32, 33]

Table (5) showed also a significant decrease in the sperm motility. This suggests that the drug was able to permeate the blood- testis barrier with a resultant alteration in the microenvironment of the semniferous tubules[34] and thus, creating a different microenvironment in the inner wall of the semniferous tubules from that in the outer parts.[35] It is known that the structure and function of the epididymis are dependent on androgens.[36] In this study, a dose related suppression of the epididymis sperm motility in treated rats suggests an under supply of testosterone to epididymis and therefore an impaired epididymal function. The impaired epididymal function may also be due to reduced activity of the testes which affects the normal passage of the testicular fluid into the epididymis.[37, 38, 39] This is also confirmed by the reduced epididymal weight. Furthermore, It has been reported that androgen binding protein and testosterone produced by the Leydig cells must reach the epididymis in a sufficient amount through the testicular fluid and maintains the epididymis testosterone level, since testosterone level affects the functional integrity of testes and epididymis, sperm concentration, motility and viability of spermatozoa.[40, 41, 42]

Sulfasalazine induced a reduction in the percentage of progressively motile sperms possibly by suppressed synthesis of sperm membrane proteins located in the acrosomal region of the sperms head which could lead later to changes in cytoplasmic calcium levels and to a decrease in sperms motility.[43]

Kato and coworkers (2002) had observed also that treatment with sulfasalazine caused a remarkable decrease in sperms velocity and an increase in the beat frequency of the sperm head which were considered as main causes of infertility.[44] This is in consistent with that observed by Ohashi et al (1995) who indicated that a reduction in acrosome reaction ratio is one of the mechanisms of induction of decreased fertility by sulfasalazine as well as the depressed percentage of progressively motile sperms.[45] This study referred to highly significant decrease in sperm viability with increasing the dose of diazepam (table 5), this is in consistent with that indicated by Mohana et al. (2013) who found that diazepam decreased significantly the motility and viability of goat epididymal sperms.[46] They suggested that diazepam induced oxidative stress in goat epididymal sperms and that it had a significant role in disturbing the balance between oxidative stress and antioxidant system. They concluded that the use of diazepam could be a considerable factor in causing infertility in human males. Table (5) showed also a significant increase in the percentage of morphologically abnormal sperms this may be due to the drug interference with the spermatogenic processes in the

semniferous tubules, epididymal functions and testosterone activity on the hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in alteration of spermatogenesis.[47,48] Kar and Das (1983) demonstrated a significant incidence of abnormalities induced by diazepam on sperms involving both shape and size of the sperm head and tail.[49] This is in consistent with our study which had shown a significant increase in sperm abnormalities.

Figure (1) showed normal morphologically and structurally tissue with normal components of germinal epithelium that lining the semniferous tubules which include normal spermatogenic lineage cells (spermatogonia, spermatocyte and spermatid); the spermatogonia are attached to the basal lamina, while the primary spermatocytes are larger than the spermatogonia and occupies the middle zone of the germinal epithelium, but the secondary spermatocytes are about half size of the primary spermatocytes and lie nearer the lumen. The spermatids lies close to the lumen and are spherical or polyglonal cells. And the supporting cells (Sertoli cells) are surrounded by thin connective tissues.[50]

Figure (2) showed some of the semniferous tubules clear and empty in the 2mg/kg of diazepam treated rats, compared to the crowded semniferous tubules filled by spermatozoa in the control group. The above findings are more pronounced with increasing the dose of diazepam (5 mg/kg); figure (3) in which the spermatogonia in the semniferous epithelium of the diazepam treated groups were over-populated most of the other spermatogenic cells and thus, there is a relative preponderance of primitive germ cells on the expense of more mature ones. The reduction or loss in number of primary spermatocytes and secondary spermatocytes and spermatids in treated rats with diazepam reflected the non-avaibility of androgen binding protein (ABP) from sertoli cells.[51] ABP is required to maintain intra- testicular androgen concentration and transformation of advance stages of germ cells. Meiotic and post- meiotic germ cells were highly sensitive to androgen concentration.[52, 53]

Figure (4) showed a cross section of rat´s testis treated with 10mg/kg of diazepam, it showed degeneration and necrosis among spermatogenic cells in the semniferous tubules and decreasing in number of sertoli cells also the semniferous tubules were shrink distorted containing detached spermatogenic cells and few numbers of sperms and leydig cells. The atrophic state of Leydig cells in the testes of treated animals may be due to declined LH secretion.[54,55] A reduction in the number of sertoli cells will adversely affect

spermatogenesis as sertoli cells provide all or most nutritional and physical support for the developing germ cells.[56]

Figure (5) showed cross sections of rat´s testis treated with sulfasalazine; showed different damage of the semniferous tubules such as disorders of the systemic arrangement of the stages of spermatogenesis, loss of one or more stages of spermatogenesis and few numbers of leydig cells with few numbers of sperms. The RT-qPCR analysis showed by this study indicated that the StAR mRNA expression was decreased with a corresponding increase in the dose of diazepam, (table 5).

StAR is necessary for the transfer of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane in Leydig cells and this step is the rate-limiting regulated step in steroidogenesis.[57, 58] The inhibition of StAR expression results in a dramatic decrease in steroid biosynthesis.[59] In this study, expression levels of StAR were significantly decreased following exposure to 5 or 10 mg diazepam/kg. This reduction may affect cholesterol delivery across the mitochondrial membrane and thus contribute to the decrease in testosterone synthesis.[60]

Several mechanisms underlying the reduction in mRNA expression of StAR gene caused by the drug closely correlated with the fact that Ca affects the transfer of cholesterol to the inner mitochondrial membrane, the rate – limiting step in steroidogenesis.[61,62] This was confirmed by a study reporting a $Ca2^{+}$ induced increase in StAR protein[13], which is critical for the cholesterol transfer to the inner mitochondrial membrane to initiate steroidogenesis.[63,64,65]

As $Ca2^{+}$ ions are required for several steps of steroidgenesis, diazepam as $Ca2^{+}$ channel antagonist would be expected to have an effect on StAR protein expression.

StAR expression is stimulated via LH binding to its receptor (LHR). Therefore, LH also plays an indirect role in the delivery of cholesterol to the inner mitochondrial membrane.[66] This study revealed a significant reduction in serum LH levels in rats exposed to 5mg and 10 mg/kg. These results suggest that a reduction in LH results in decreased StAR expression at levels of 5mg and 10 mg of diazepam/kg and therefore, diazepam affects the transport of cholesterol into the inner mitochondrial membrane.[67]

CONCLUSION

Diazepam in a dose dependent pattern was effective in attenuating steroidogenesis and testosterone production through its inhibitory effects on StAR protein gene expression in rats.

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