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The Effect of Silymarin on Spermatogenesis of Male Quails Exposed to Heat Stress

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Abstract

Many factors could impact spermatogenesis. The influence of silymarin on some sexual efficiency, Sertoli cell, Leydig cell, and Johnsen's score alterations in male quails exposed to thermal stress has been studied. Quail were placed into four groups of ten birds each: 1st group control, 2nd group heat-related stress at 42±2 ºC for 4 hours daily, 3rd group heat-related stress at 42±2 ºC for 4 hours daily with 200 mg/kg of silymarin orally. The 4th group received 200 mg/kg of silymarin orally. The heat-related stress group's results indicate a notable drop in sperm count and the percentage of viable sperm, testosterone hormone, luteinizing hormone, glutathione level, foam gland weight, diameter of seminiferous tubules, Sertoli cell, Leydig cell, and Johnsen's score and a rise in dead sperm percentage, abnormal sperm percentage, and MDA level relative to control values. Heat stress with the silymarin group existing a notable rise in sperm count, testosterone hormone, foam gland weight, glutathione level, diameter of seminiferous tubules, Sertoli cells, Leydig cells, and Johnsen's score and a decrease in MDA level compared with the heat stress group, the silymarin group displayed a notable rise in testosterone hormone and glutathione level versus the control group. This study concluded that silymarin has beneficial effects on male quail infertility due to heat stress.

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1. Introduction

Numerous factors can influence the motility, quantity, and DNA structure of sperm, which in turn can impact fertility. One of these important elements is oxidative stress. The plant known as milk thistle, Silybum marianum, yields a combination of flavonoids called silymarin (SM) [1]. According to silymarin's protective functions against physical stress, disorders of membrane integrity, mitochondrial functions, apoptotic, fibrotic, inflammatory, oxidant, and immunomodulatory [2]. The exposure of birds to hot environmental conditions can cause heat stress in both tropical and subtropical regions, negatively affecting their productive performance and causing significant economic losses [3]. It is well known that the poor performance of birds exposed to heat stress is primarily due to reduced feed intake to minimize metabolic heat production [4]. Exposure to a temperature of 34 degrees Celsius in laying quails led to a decrease in egg production and feed conversion efficiency, in addition to a deterioration in egg quality [5]. Also, cholesterol levels were higher in the yolk and serum, and general stress indicators improved in the quail when exposed to thermal stress [6]. Previous research has been conducted on the negative consequences of stress from heat on quail production, which has proven that the adverse effects of excessive heat impact the productivity of the birds [7]. The harmful effects of thermal stress range from a decrease in body weight by 7.7 to 13.2%, growth rate by 11.0 to 14.5%, feed intake by 6.1 to 21.6%, feed efficiency by 4.3 to 8.6%, and egg production by 6.6 to 23.3% [8]. Hormone changes are also considered essential in the complicated interactions for quails living in the environment with higher temperatures to develop defective spermatogenesis and respond to thermal stress. Through reproduction and neuroendocrine glands in quails play an essential role in controlling how their reproductive systems react to heat stress, other elements such as photoperiod may

have an impact on how well they reproduce [9]. The goal of this research is to investigate the impact of silymarin as an antioxidant on sex productiveness and testicular histological alterations in quail subjected to heat stress.

2.MATERIALS AND METHODS

2.1 Ethical approval: All scientific experiments were conducted under approval Institutional Animal Care and Use Committee / UM.VET.2024.012

2. 2 birds housing:

The birds were brought at the age of 8 weeks to the animal house of the College of Veterinary Medicine, University of Mosul, from 11/7/2024 to 1/21/2025. After acclimatizing the birds for more than a week, the birds were distributed in the closed poultry hall into four groups of ten birds each. The control group was kept at room temperature. The heat stress group was kept at an ambient temperature of 42±2°C. The third group was kept at an ambient temperature of 42±2°C and given 200 mg/kg silymarin orally. The fourth group was given silymarin orally at room temperature. The lighting was natural, the poultry hall was provided with ventilation fans, and the birds were given a balanced diet according to the Scientific Council for Nutrition, and water was given *ad libitum*.

2. 3 Silymarin

Silymarin was purchased from an NOW American company, and the dose was prepared according to the weight of the bird, as the weights of the birds ranged from 120±10 grams.

2.4 Design of experiments

Quails were split up into four categories at random, with 10 quails in each collective. 1st control group, 2nd heat stress group: the quails were exposed to heat at 42±2ºC for 4 hours daily for ten weeks [10]. 3rd heat stress with the silymarin group: the birds were exposed to heat at 42±2ºC for 4 hours daily together with oral silymarin at 200 mg/ kg of body weight for ten weeks. [11]. In the 4th silymarin group, the birds were administered 200 mg/ kg of silymarin orally.

2.5 sampling process

From the jugular vein, blood was drawn and placed in tubes devoid of anticoagulants and then placed in a centrifuge for 15 minutes to obtain serum. Testosterone and luteinizing hormone values were determined by utilizing a hormone enzyme ELISA kit (Sun Long Biotic). Following the guidelines provided by the manufacturer. Glutathione and malondialdehyde values were found by using a spectrophotometer at 540 waves

Ten birds from each group were dissected via the male quail's abdominal area at the end of of the trial period. After removing the foam gland and both testes, the relative weight of each animal was determined. Eosin stain was used in a Petri dish to determine the sperm concentration after the vas deferens were isolated in order to count and assess the sperm analysis. A drop of eosin stain and a drop of nigrosine stain were used to determine the proportion of live, dead, and malformed sperm. To determine the percentage of aberrant sperm, a drop was placed on a slide and examined at 100x magnification using an oil immersion lens.

After performing the anatomical examination of the birds, the testis was taken and placed in laboratory containers containing a 10% neutral buffered formalin solution to be fixed for 3 days. Subsequently, histological sectioning was performed to calculate the Sertoli cells count, Leydig cells count, and Johnsen's score

2.7 Statistical analysis

Data were represented as mean ± ES, the significance of the variations among the different groups was tested, and the data were statistically analyzed using one-way ANOVA. Then followed by the Duncan's multiple range assay with a $P \leq 0.05$ significance level by using of the SPSS software V.25.

3. Results

3.1 Impact of silymarin on spermatogenesis in quail birds exposed to heat stress:

We observe from Table (1) a notable drop ($P \leq 0.05$) in sperm count in the group under heat stress 42 ±2 °C, in contrast to normal control values. On the other hand, in the condition of heat stress with the silymarin group, the results showed a notable rise in sperm concentration related to the heat stress values. The results in the table 1 indicate no significant difference in sperm count in the silymarin group, in contrast with the control group values. The percentage of live sperm in the heat stress group was significantly lower than in the control group, according to the results of Table 1. In heat stress with the silymarin group, the results did not show a notable difference in live sperm percentage relative to the heat stress values. Similarly, the results did not show a difference in live sperm percentage in the silymarin group, opposite to the control values. The findings showed that male quail affected by heat stress significantly raised the percentage of dead sperm. However, in heat stress with the silymarin group, the results showed no significant difference in the number of sperm that die relative to the heat stress values. The data of current research showed that treating male quails with silymarin did not lead to a difference in dead sperm, in the contrast to the control values. Exposure to thermal stress caused the proportion of sperm with deformities to rise in comparison to the control group. In the heat stress with silymarin group, the results indicated no significant difference in the percentage of deformed sperms relative

to the heat stress group. Additionally, the findings revealed no discernible variation in the malformed sperms in the silymarin group.

Table 1. The influence of silymarin on spermatogenesis in male quails under heat stress.

Treated	Sperm count 10^6 /ml	Live sperm %	Dead sperm%	Abnormal sperm %
Control group	2.12±0.06 a	76.33±3.27 a	22.16±4.00 b	0.50±1.50 b
Heat stress 42±2°C group	1.26±0.14 b	23.33±2.60 b	68.00±1.86 a	1.20±8.66 a
Heat stress 42±2°C with silymarin 200 mg/kg group	2.29±0.17 a	30.00±1.96 b	64.00±2.87 a	1.07± 6.16 a
Silymarin 200 mg/kg group	2.69±0.33 a	75.89±4.06 a	22.00±4.16 b	0.60±2.16 b

Data was expressed as mean ±SE, at a probability level of less than or equal to P≤0.05, and distinct small letters inside the identical column reveal significant variations among the groups.

3.2 Impact of silymarin on serum testosterone, and luteinizing hormone and the weight of the foam, testis in male quails under heat stress.

As noticed in Table (2), the heat stress group's the testosterone hormone values significantly declined (P≤0.05) when exposed to a temperature of 42ºC versa to normal control values, but the heat stress with silymarin group's testosterone hormone was substantially higher in comparison to the heat group. The findings also showed that, in comparison to the control group, silymarin significantly raised the testosterone levels in the silymarin group. According to the Table 2 findings, the heat stress group's luteinizing hormone level significantly declined, whereas the heat and silymarin groups' luteinizing hormone levels did not change significantly from the heat group. We find no significant difference in the levels of luteinizing hormones between the silymarin group and the control group. According to the findings in Table 2, the weight of the foam gland significantly declined as a result of heat stress exposure in comparison to the control group. The weight of the foam gland elevated significantly in the heat stress with silymarin group compared to the heat group, but the Table 2 findings demonstrated in apparent that silymarin had no discernible effect on the weight of the foam gland in the silymarin group. The weight of the right and left testes of the heat stress group did not change significantly (P>0.05), according to the study's findings. Similarly, in the group exposed to heat stress with silymarin, the results did not show a significant difference (P>0.05) in the weight of the right and left testes when compared to the heat group. In the silymarin group, the table data did not show a significant difference (P>0.05) when compared to the control group.

Table 2. The influence of silymarin on serum testosterone and luteinizing hormone, and the weight of the foam, testis in male quails under heat stress.

treated	Testosterone hormone (nanograms/mL)	luteinizing hormone (nanograms/mL)	Foam gland weight g/100g of B. W	Right testis weight (g)	Left testes weight (g)
Control group	3.36 ± 0.15 b	1.25 ± 0.14 A	1.55±0.09 a	1.81 ± 0.15 a	1.51 ± 0.16 a
Heat stress 42±2°C group	2.65 ±0.11 c	0.84 ± 0.06 B	0.76±0.04 b	1.68 ± 0.04 a	1.37 ± 0.15 a
Heat stress 42±2°C with silymarin 200 mg/kg group	3.39 ± 0.20 b	1.15 ± 0.06 Ab	1.37±0.08 a	1.73± 0.13 a	1.45 ± 0.16 a
Silymarin 200 mg/kg group	4.66 ± 0.13 a	1.24 ± 0.16 A	1.49±0.08 a	1.78 ± 0.15 a	1.88 ± 0.22 a

Data were expressed as mean ±SE, at a probability level of less than or equal to P≤0.05, and distinct small letters inside the identical column reveal significant variations among the groups.

3.3 Impact of silymarin on glutathione and malondialdehyde levels in the serum of male quails under heat stress

The findings in Table 3 demonstrated that when the birds were exposed to a temperature of 42 ±2 ºC, their glutathione levels significantly dropped in the heat stress group as opposed to the control group. The glutathione level was significantly

higher in the silymarin with heat stress group than in the stressed group**.** When compared to the control group, the data showed a substantial rise in glutathione value in the silymarin group. Malondialdehyde levels in the heat stress group were significantly higher than in the control group, as shown by the data. The data revealed a significant drop in malondialdehyde levels in the silymarin with heat stress group versus the heat stress group. The levels of malondialdehyde in the silymarin group and the control group did not differ significantly, as determined by the data.

Table 3. The influence of silymarin on serum glutathione and malondialdehyde levels in male quails under heat stress.

Effect of silymarin on of male quails under heat stress.

treated	Glutathione (micromole /L)	Malondialdehyde (micromole /L)
Control group	4.25 ± 0.25 B	0.58 ± 0.04 b
Heat stress 42±2°C group	2.05 ± 0.15 C	0.82 ± 0.05 a
Heat stress 42º±2°C with silymarin 200 mg/kg group	3.98 ± 0.25 B	0.47 ± 0.04 b
Silymarin 200 mg/kg group	5.03 ± 0.17 A	0.43 ± 0.07 b

Data were expressed as mean ±SE, at a probability level of less than or equal to P≤0.05, and distinct small letters inside the identical column reveal significant variations among the groups.

3.4 Impact of silymarin on the diameter of seminiferous tubules, Sertoli cells count, Leydig cells count, and Johnsen's score in male quails under heat str

As shown in Table 4, male quail exposure to heat stress led to a significant reduction in the diameters of the seminiferous tubules, as illustrated in Figure 2, when compared to the control group, as shown in Figure 1. The diameters of the seminiferous tubules, as demonstrated in Figure 3, were significantly larger in the heat stress with silymarin group than in the heat stress group. The statistics showed that the silymarin group as illustrated in Figure (4) did not differ significantly from the control group. Based on the findings, heat stress significantly reduced the number of Sertoli cells Figure 2 in comparison to the control group. The number of Sertoli cells Figure 3 was significantly higher in the heat stress with silymarin group than in the stress related heat group, silymarin did not cause a significant variation in the number of Sertoli cells, as shown in Figure 4, when compared to the control group. According to the findings, the heat stress group's Leydig cell counts Figure 2 were significantly reduced in comparison to the control group, this was accompanied by a significant increase in Leydig cells number Figure 3 in male quails under heat stress with silymarin group compared to the heat stress group, however the results did not lead to any occurrence a significant difference was observed in the Leydig cells count in the silymarin group Figure 4. The results indicated that continuous exposure to high temperatures caused a significant decrease in the Johnson score in heat stress group, while heat stress with silymarin group caused elevated in Johnson score when compared to the stress-related heat group, the results presented in the table indicated that treating the birds with silymarin did not produce a statistically significant variation than the control group

Table 4. The influence of silymarin on the diameter of seminiferous tubules, Sertoli cells count, Leydig cells count, and Johnsen's score in male quails under heat stress.

Treatment	Diameter of seminiferous tubules (µm)	Sertoli cells count	Leydig cells count	Johnsen's score
Control group	412.6 ± 34.7 a	5.80 ±0.32 a	3.9 ± 0.37 a	8.5 ± 0.26 a
Heat stress at 42±2°C group	278.8 ± 24.1. b	3.5 ± 0.34 b	2.8 ± 0.29 b	4.4 ± 0.32 c
Heat stress at 42ºC with silymarin 200 mg/kg group	383.8 ± 16.2 a	5.1 ± 0.37 a	3.7 ± 0.3 a	7.2 ± 0.4 b
Silymarin addition 200 mg/kg group	420.1 ± 36.5 a	6.0 ± 0.39 a	4.0 ± 0.25 a	8.8 ± 0.32 a

Data were expressed as mean ±SE, at a probability level of less than or equal to P≤0.05, and distinct small letters inside the identical column reveal significant variations among the groups.

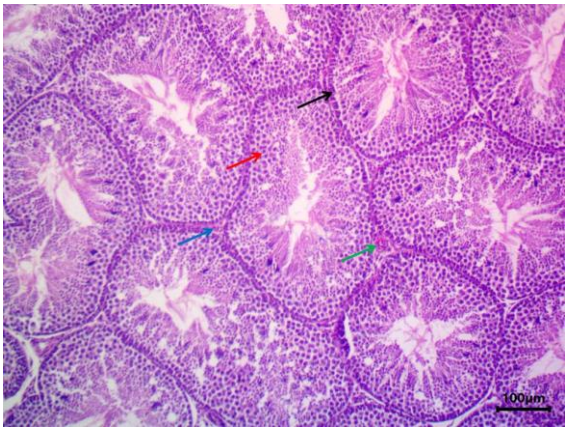

Figure 1: Histological section of quail's testis of the **control group G1** shows intact seminiferous tubules with normal thickness of epithelium (→), active spermatogenesis and intact spermatogenic cells (→), interstitial tissue (→) and blood vessels (→). H&E stain, 100X

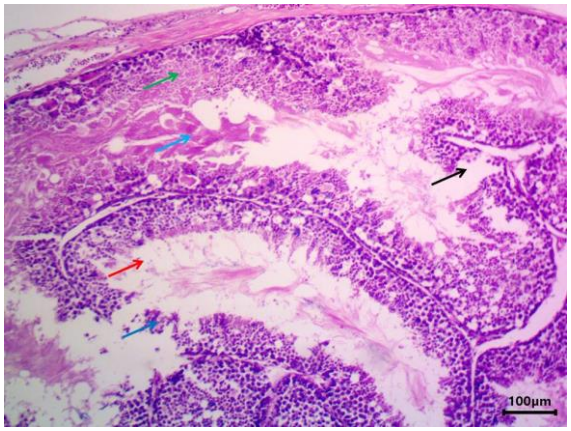

Figure 2: Histological section of quail's testis of the **heat stress group G2** shows disorganization of seminiferous tubules (→) impairment spermatogenesis as no spermatozoa in wide lumen seminiferous tubules (→), decreased the spermatogenic cells layer thickness (→) with necrosis of spermatogenesis cells (→) and edema (→). H&E stain, 100X.

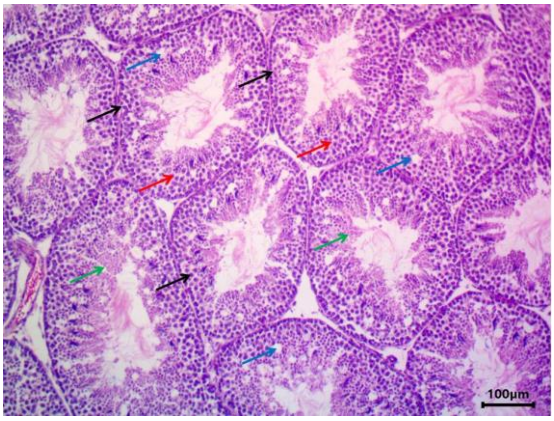

Figure 3: Histological section of quail's testis of the **heat stress with silymarin treatment group G3** shows intact histological structure of seminiferous tubules (→), intact spermatogenic cells layer thickness (→), degeneration of spermatogenesis cells (→) and mild necrosis (→) and edema (→). H&E stain, 100X.

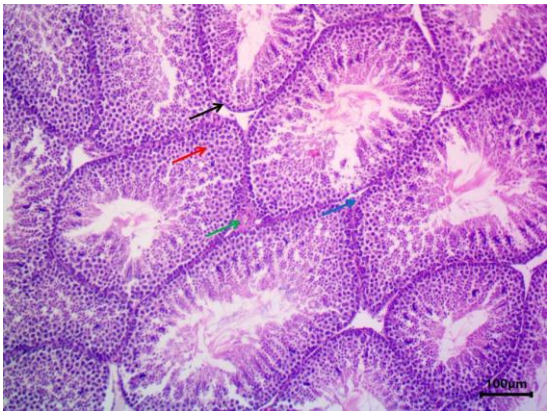

Figure 4: Histological section of quail's testis of the **silymarin treatment group G4** shows intact seminiferous tubules with normal thickness of epithelium (→), active spermatogenesis and intact spermatogenic cells (→), interstitial tissue (→) and blood vessels (→). H&E stain, 100X.

4. Discussion

The data of the present study indicated a decrease in sperm count, number of live sperms, testosterone and luteinizing hormone levels, and an increase in dead and abnormal sperm percentage in the group exposed to heat stress. This finding is comparable to another study where crowding-induced stress resulted in a drop in testosterone concentration, normal sperm count, and live sperm count while raising the percentage of dead and malformed sperm in male quails [12]. The reason for this effect may be attributed to the decrease in luteinizing hormone, which resulted in testosterone levels dropping, and this is what our current study has proven. These findings aligned with an earlier study that indicated exposure to high temperature in male quails resulted in a decline in testosterone and luteinizing hormone [13]. This result may be explained by the fact that high temperatures stimulate the hypothalamus, which in turn affects the pituitary gland by secreting luteinizing hormone [14].

Another study confirmed that stressful factors lead to a reduction in the number of luteinizing hormone receptors in Leydig cells, resulting in a decrease in testosterone synthesis [15]. and the sperm count, and may decrease the number of the live sperm and abnormal sperm. The present study's findings indicated that being subject to high temperature with silymarin led to an increase in sperm count. This outcome was acceptable with the findings of a prior study that revealed the effect of silymarin on sperm counts [16]. The causative factor of this result is that stress induced by high temperature can cause an excessive formation of reactive oxygen and nitrogen species (ROS + RNP), which are harmful to spermatogenesis [17]. Furthermore, it has been demonstrated that silymarin functions as a powerful antioxidant, neutralizing reactive oxygen species and preventing lipid peroxidation, thus defending cells from oxygen radicals [18]. Antioxidants may therefore be a helpful management strategy to increase male fertility [19]. Therefore, silymarin, as an antioxidant, overcomes the negative consequences of heat stress. Our present data showed that silymarin didn't cause defective spermatogenesis; this result disagrees with a previous study that showed silymarin enhances the number of sperms [20]. Perhaps the reason is the difference in dose, length of time given, animal species and conditions surrounding the experiment. The study results indicated exposure to high temperature with silymarin caused elevated in testosterone levels and no significant difference in luteinizing hormone levels, these findings matched with those from different research that showed the impact of silymarin on testosterone levels in animals exposed to stress [21], this is what our current study has proven through histological testes tissue sections, which revealed that the Leydig cells count had significantly increased. According to our findings, silymarin stimulates the secretion of the testosterone hormone and increases its levels This outcome aligned with a prior study that demonstrated milk thistle raised testosterone values in adult quails [22]. This process is attributed to the flavonoids present in milk thistle, which increase the transcription of steroid-regulating proteins, thereby raising testosterone levels in the testes of male quails [23]. The current result showed there were no changes in luteinizing hormone level in the silymarin group, and this result was in correlation with a previous study showing silymarin didn't cause differences in luteinizing hormone [24]. Maybe the route of administration, and the period of giving the silymarin, is one of the causes that didn't significantly affect. According to the current study's findings, quails subjected to heat stress showed a reduction in foam gland weight, and this was in line with a prior study on stressed quails that showed a drop in the weight of the foam gland [25]. This is due to oxidative stress, which promotes tissue damage in reproductive organs under heat stress [26]. The data of the current study display that heat stress with silymarin causes an increase in foam gland weight, and this result was in line with another study, which indicates the role of silymarin in enhancing the performance body in broilers under summer conditions [27]. The reason for this increase may be due to the elevation in testosterone level and its relation to the foam gland. The weight of the left and right testes did not differ, according to the data of male quails across all groups. These results disagree with another study [28]. Our research demonstrated that male quails exposed to heat stress had lower glutathione and higher levels of malondialdehyde. These findings aligned with a prior study that documented a reduction in glutathione levels in white laying hens subjected to high temperature stress [29]. They also concurred with a second study on chickens that showed that extreme temperatures significantly raised the levels of malondialdehyde [30]. The reason for these decreases when excessive heat being subjected is linked to its effect on the oxidative balance in the body, causing the accumulation of ineffective oxygen and a reduction in oxidative enzymes leading to oxidative stress in the body [31], when exposed to heat stress for longer than three days, reactive oxygen species formation increases and impacts enzyme function, which lowers glutathione and raises malondialdehyde activity [32].

According to the current study's findings, when the silymarin group was exposed to heat stress, glutathione levels rose and malondialdehyde levels dropped. These results were in line with those of a study on broiler chicks that found that glutathione levels rose while malondialdehyde declined [33]. This finding suggests silymarin's antioxidant function in prevention. The oxidation of lipids results from a rise in ROS generation under heat stress conditions, which elevates MDA level and impairs the endogenous defense system by reducing antioxidant enzyme involved glutathione peroxidase, silymarin scavenging free radical and inhibiting lipid peroxidation and reduces MDA, also enhance the endogenous antioxidant by activate the Nrf2(define the symbol) signaling pathway which elevate glutathione level [34]. Our study indicated that silymarin led to an increase in glutathione levels with no difference in malondialdehyde levels. This outcome aligned with the results of an earlier investigation into broiler chickens [35]. This increase is due to the action of silymarin in increasing hepatic glutathione; additionally, it has been demonstrated that silymarin improves hepatocytes' capacity to recognize proteins via promoting RNA polymerase activity

I [36]. The current study's demonstrated that exposure to heat stress significantly reduced the diameter of the seminiferous tubule Sertoli cell counts, Leydig cell counts, and Johnsen's score in heat stress group these outcomes were in line with those of a prior study on chicks that showed exposure to heat stress significantly reduced the diameter of the seminiferous tubules [37], and aligned with those of a prior study that identified a reduction in Sertoli and Leydig cell counts in geese subjected to heat stress [38] this reduction may be due to the result of our study also, which indicated decrease in testosterone and LH hormones level in heat group because the relation between testosterone and cell in maintenance the structure and physiological functions of these cells. Sertoli cell counts, Leydig cell counts, and Johnsen's score were significantly increased after being exposed to heat stress with silymarin, according to the present study. And this outcome was consistent with a prior study that showed silymarin increases the number of Sertoli and Leydig cells in oxidative stressors as well as the diameter of the seminiferous tubules [39]. To explain this increase is due to the protective mechanism of silymarin as an antioxidant [40]. It has been demonstrated that silymarin improves the hypothalamic-pituitary-gonadal (HPG) axis by dramatically raising serum levels of testosterone and luteinizing hormone, which in turn directly stimulate the testes' Sertoli and Leydig cells, improving the spermatogenesis mechanism. [41]. The current study's findings demonstrated that silymarin had no discernible effect on the diameter of seminiferous tubule, Sertoli, and Leydig cells. These findings were in line with those of a study that showed silymarin had no discernible effect on the diameter of seminiferous tubule, Sertoli and Leydig cells [20].

5. Conclusions

Our results indicate the antioxidant functions of silymarin in the improving the physiological parameters, including spermatogenesis under heat-stressed growing quail, by enhancing antioxidant capacity, reducing oxidative damage, and maintaining testicular structure and functions.

6. Conflict of Interest

The researchers have declared no conflicts of interest.

7. Acknowledgements

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تأثير السيليمارين على تكوين النطف في ذكور السمان المعرضة للإجهاد الحراري

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الخلاصة

هناك العديد من العوامل التي قد تؤثر على تكوين الحيوانات المنوية وفعاليتها. دُرست تأثيرات السيليمارين على بعض التغيرات في كفاءة تكوين النطف وفعاليتها وخلايا سيرتولي، وخلايا لايديك، ومقياس جونسن لدى ذكور السمان المعرضة للإجهاد الحراري. وُضعت طيور السمان في أربعة مجاميع، كل مجموعة تضم عشرة طيور: المجموعة الأولى السيطرة، المجموعة الثانية إجهاد حراري عند درجة حرارة 42±2 درجة مئوية لمدة 4 ساعات يوميًا، المجموعة الثالثة إجهاد حراري عند درجة حرارة 42±2 درجة مئوية لمدة 4 ساعات يوميًا، مع جرعة 200 ملغم/كغم من السيليمارين عن طريق الفم. المجموعة الرابعة تلقّت جرعة 200 ملغم/كغم من السيليمارين عن طريق الفم. تشير نتائج مجموعة الإجهاد الحراري إلى انخفاض معنوي في عدد النطف ونسبة النطف الحية وهرمون التستوستيرون والهرمون اللوتيني في مصل الدم، ومستوى الكلوتاثايون ووزن الغدة الرغوية وقطر االنبيبات المنوية وعدد خلايا سيرتولي وخلايا لايديك ومراتب جونسن وارتفاع في نسبة النطف الميتة ونسبة النطف غير الطبيعية ومستوى MDA مقارنةً بقيم السيطرة. أظهرت مجموعة الاجهاد الحراري مع السيليمارين ارتفاعًا معنويا في عدد النطف وهرمون التستوستيرون ووزن الغدة الرغوية ومستوى الكلوتاثايون وقطر النبيبات المنوية وعدد خلايا سيرتولي، وخلايا لايديك ومراتب جونسن وانخفاضًا في مستوى MDA مقارنةً بمجموعة الإجهاد الحراري، بينما أظهرت مجموعة السلييمارين ارتفاعًا معنويا في هرمون التستوستيرون ومستوى الكلوتاثايون مقارنةً بمجموعة السيطرة. وخلصت هذه الدراسة إلى أن السيليمارين له آثار مفيدة على عقم ذكور السمان الناتج عن الإجهاد الحراري.