



The Role of Silymarin in Improving Physiological Markers Related to Heat Stress in Male Quails

Nabaa Faris Nayef and Ashwaq Ahmed Hassan

Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine,
University of Mosul, Mosul, Iraq

ABSTRACT

The purpose of the research was to investigate the ability of silymarin to minimize the effects of thermal stress on physiological markers in male quails. Forty adult male birds, weighing 120 ± 10 g age, 8 weeks, were randomly allocated into four groups. Control group. Heat stress group: the quails exposed to a temperature of $42 \pm 2^\circ\text{C}$ for 4 hours daily. Heat stress with the silymarin group: the quails were exposed to a temperature of $42 \pm 2^\circ\text{C}$ for 4 hours daily while receiving 200 mg/kg of silymarin orally. Silymarin group: the quails receiving 200 mg/kg of silymarin orally. The experiment lasted ten weeks. Heat stress reduces WBC, RBC, PCV, Hb, MCV, MCH, MCHC, lymphocyte%, HDL, liver, and body weight while raising the heterophil/lymphocyte ratio, heterophil%, ALT, AST, ALP, HSP70, cholesterol, and triglyceride levels in contrast to the control group. Heat stress with silymarin elevated WBC, RBC, Hb, MCV, MCH, MCHC, lymphocyte%, and HDL while diminishing H/L ratio, heterophil%, ALT, AST, ALP, HSP70, and triglyceride linked with the heat stress group. Silymarin group had higher WBC, lymphocyte%, HDL and lower the H/L ratio, heterophil%, and ALT values compared to the control group. The study identified that silymarin enhanced physiological markers in male Japanese quails under thermos-stressed conditions.

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E-mail addresses:

dr.ashwaqah@uomosul.edu.iq

ORCID: 0000-0003-4883-6192

* Corresponding author

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INTRODUCTION

One of the substances the milk thistle plant has in its leaves and fruits is silymarin, which is made up of five different polyphenolic families. Among which, silybins are the most potent, followed by isosilybins, silcristin, silydianin, isosilychristin, and one flavonoid (taxifolin) [1]. Silymarin, a naturally occurring substance found in the seeds and leaves of the milk thistle plant, is known as an antioxidant and has been used to treat liver disorders [2]. In the liver, silymarin acts by decreasing glutathione oxidation, raising

antioxidant activity, and promoting ribosomal polymerase synthesis to regenerate and multiply its cells [3]. In addition to its beneficial effect on the liver, silymarin may also have benefits for the heart, kidneys, and neurological system. It also possesses antiviral, antioxidant, anti-inflammatory, and anti-fibrotic qualities throughout the body [4].

Silymarin is used to treat disorders of the kidneys, gallbladder, skin, lungs, and prostate in addition to neurological conditions [5]. Silymarin can be utilized in intense chicken production systems to stimulate the growth of broilers and to increase the number and size of eggs. It can be utilized as a hepatonic to enhance the immune system of poultry, to improve intestinal health, and to improve the carcass qualities of broiler chickens [6]. Supplementing with silymarin enhanced broiler growth, decreased oxidative stress, enhanced the quality of the meat, raised unsaturated fatty acids, and lessened the impacts of mycotoxin [7]. Due to its abundance of flavonolignans, silymarin possesses anti-inflammatory immune-stimulating, and antioxidant properties [8].

Heat stress has severely affected the health of animals and caused large financial losses in the production of public hygiene and farm animal services apparent [9]. Moreover, if environmental changes worsen, the detrimental impacts of thermal stress will become more apparent [10] is regarded as one of the conditions that adversely impacts broiler and layer chicken production, with results ranging from lower growth and egg output to a decline in poultry quality [11]. The lymphocyte count, antibody formation, lymphocyte proliferation, cytokines, and immunoglobulin concentration are all impacted by heat stress, as is immunity [12]. Because they normally maintain balance in the animal's body through constant depletion and compensation, red blood cells and immune cells are a crucial sign of how the immune

system is impacted by thermal stress [13]. The body's immunological response is typically impacted by heat stress, as evidenced by the fact that laying hens subjected to heat stress have been shown to have decreased thymus and spleen weight [14]. The goal of the research is to determine the effect of silymarin administration on the physiological parameters of male quails under heat stress.

MATERIAL AND METHODS:

Ethical approval:

All scientific experiments were conducted under approval by Institutional Animal Care and Use Committee number / UM.VET.2024.012

Japanese quail diet and housing:

The research was conducted at the Animal House of the Faculty of Veterinary Medicine, University of Mosul, Iraq. Forty birds, age 8 weeks weighing approximately 120 ± 10 grams for a 10 weeks duration of treatment from 7/11/2024 to 21/1/2025 were used. The quails were allowed a one-week acclimatization period before the experiment began. The Japanese quails were randomly distributed in a closed poultry hall that was divided into several rooms, each measuring 1.5×1 m².

The first room, the control group, was set at 25°C. The second room, the heat stress group, was equipped with an electric heater and a thermostat. When the temperature reached 42°C, the birds were put in the room for four hours daily. The third room, the heat stress group with silymarin, was also equipped with an electric heater and a thermostat. When the temperature reached 42°C, the birds were put in the room for four hours daily, while the birds were administered 200 mg/kg of silymarin orally daily. The fourth room, the silymarin group, was set at 25°C, and the birds

were administered 200 mg/kg of silymarin orally daily. Also, the poultry halls were equipped with ventilation fans and 10 hrs of natural light and 14 hrs of darkness. The basic diet was established to keep quail's nutritional needs maintained based on NRC [15]. Water and food provided ad libitum.

Preparation silymarin:

Silymarin was received from NOW American company. Silymarin given orally by using special syringe at dose 200 g/kg. and the dose was reconstituted by thawing 200 mg of silymarin in 25 mL of distilled water and the dose calculated based on the bird's body weight.

Experimental design:

Forty Japanese male quails were equally distributed into 4 groups, with 10 birds per group. 1st control group, 2nd heat stress group, 3rd heat stress with silymarin group, 4th silymarin group.

Heat stress protocol:

Heat stress group: birds were subjected to a temperature of $42\pm 2^{\circ}\text{C}$ from week 8 to 18, for four hours from 9:30 am to 1:30 pm daily, as mentioned by Hassan and Asim [16].

Heat stress with the silymarin group: quails were subjected to a temperature of $42\pm 2^{\circ}\text{C}$ from weeks 8 to 18, for four hours from 9:30 am to 1:30 pm daily. Additionally, quails were given 200 mg/kg silymarin orally daily during the experiment, as mentioned by Fathi et al. [17].

Blood collection and hematological tests:

After 7 weeks of the experiment, quails were hand-restrained, and blood was collected from the jugular vein using a special needle. Blood samples were collected in the morning

(at 8.00 to 9.00 h) in an EDTA tube to get blood for complete blood picture tests to determine total leucocyte count and total erythrocyte count according to the Natt and Herrick technique by using a hemocytometer. PCV% was calculated using the microhematocrit technique, cyanmethemoglobin concentration was calculated using Drabkin's kits at 540 nm wavelength [18], MCV, MCH, and MCHC equations were calculated, and the DLC count was obtained from a blood film using the Wright staining technique and heterophil/lymphocyte ratio counting to obtain the stress index.

Blood collection and biochemical tests:

After 10 weeks, at the end of the experiment, blood samples were collected during slaughter using Eppendorf tubes. The blood was centrifuged for 15 min at 3000 rpm, then the sera was extracted from the blood and stored at -20°C for biochemical analysis, including ALT (Biolabo), AST (Biolabo), ALP (Biolabo), HSP70 (Sun Long Biotic), total protein (Biolabo), albumin (Biolabo), globulin (Biolabo), cholesterol (Biolabo), triglyceride (Biolabo), and high-density lipoprotein (Biolabo).

Final live weight estimate:

After 10 weeks, the quails' live weights were taken. Quails were slaughtered, and the liver weight per 100 grams of body weight was calculated [19].

Statistical analysis:

The data of the current research analyzed complete blood count and laboratory biochemical indicators utilizing one-way analysis of variance (ANOVA) with Duncan multiple range test ($P < 0.05$) was considered significant [20] using the SPSS program.

RESULTS

Table 1 shows a substantial decrease ($P < 0.05$) in leucocyte count in the heat stress group relative to the unaffected control group. The heat stress with the silymarin group demonstrated a significant rise ($P < 0.05$) in WBC count in contrast to the heat stress group and returned values close to the untreated control data. The silymarin group reported a substantial elevation ($P \leq 0.05$) in WBC count when contrasted with the control values. The result of the heat stress group recorded a significant reduction ($P \leq 0.05$) in erythrocyte number linked to the normal control values. The heat stress with the silymarin group induces a substantial rise ($P \leq 0.05$) in RBC counts associated with the heat stress group. No notable changes in erythrocyte count in the silymarin group compared with the control

group. Quails exposed to heat stress produce much lower percentages of hematocrit relative to control values. Administration of silymarin 200 mg/kg B.W. to the heat-stressed quails didn't show a major effect in hematocrit value related to the heat stress group; the silymarin group's hematocrit value didn't alter appreciably ($P > 0.05$) when compared to the control values. Research data demonstrated a high decrease ($P \leq 0.05$) in hemoglobin level in the heat stress group matched to the control values. Heat stress with the silymarin group displayed a significant increase in Hb concentration relative to the heat stress group. The silymarin group produced no notable alteration ($P > 0.05$) in Hb concentration, and this result was within the normal control range.

Table 1. Role of silymarin on complete blood count of male quails exposed to heat stress (Mean \pm SE):

Treated	White blood cell $\times 10^3$	Red blood cell $\times 10^6$	Packed cell volume %	Hemoglobin Concentration gm/100ml
Control group	1.42 \pm 0.35 b	2.29 \pm 0.06 a	48.00 \pm 0.36 a	15.84 \pm 0.66 a
Heat stress 42°C group	1.27 \pm 0.25 c	1.70 \pm 0.12 b	42.66 \pm 0.71b	13.20 \pm 0.47b
Heat stress 42°C with silymarin 200 mg/kg group	1.50 \pm 0.77 b	2.17 \pm 0.19 a	43.66 \pm 0.91 b	14.60 \pm 0.50 a
Silymarin 200 mg/kg group	1.73 \pm 0.83 a	2.53 \pm 0.25 a	46.00 \pm 0.81 a	14.50 \pm 0.42 a

Different lowercase Letters in English in the identical column show a significant variance between the groups at a probability level of less than or equal to $P \leq 0.05$.

Table 2 demonstrates that the heat stress group had significantly lower MCV, MCH, and MCHC values, as well as a significantly higher stress index (H/L ratio) than the control group. The heat stress with the silymarin group had significantly elevated ($P \leq 0.05$) MCV, MCH, and MCHC levels, and a substantial

drop in stress index compared to the heat stress group. Data on the silymarin group showed no noticeable difference in the MCV, MCH, and MCHC when compared to the control group. The stress index reduced substantially in the silymarin group compared to normal control values.

Table 2. Role of silymarin on Wintrob's indices and stress index in male quails exposed to heat stress (Mean \pm SE):

Treated	MCV (fL)	MCH (Pg)	MCHC g/100ml	Stress index
Control group	217 \pm 1.00 a	68.2 \pm 1.65 a	33.1 \pm 1.35 a	1.03 \pm 0.07 b
Heat stress 42°C group	166 \pm 8.91 b	49.5 \pm 2.62 b	29.8 \pm 0.75 b	2.01 \pm 0.22 a
Heat stress at 42°C with silymarin 200 mg/kg group	209 \pm 1.50 a	66.8 \pm 3.76 a	33.0 \pm 1.25 a	0.94 \pm 0.03 b
Silymarin 200 mg/kg group	207 \pm 1.96 a	65.4 \pm 3.46 a	33.6 \pm 1.57 a	0.54 \pm 0.09 c

Different lowercase letters in the identical column show a significant variance between the groups at a probability level of less than or equal to $P \leq 0.05$.

Table 3 reveals that the heat stress group had a significantly lower lymphocyte percentage in comparison to the control group. The heat stress with the silymarin group indicated a noticeably greater lymphocyte percentage versus the heat-stressed group. The silymarin group exhibited a substantial rise in lymphocyte percentage relative to the control group. The heat stress group caused a significant increase ($P \leq 0.05$) in heterophil percentage compared to the control group. The heat stress with the silymarin group displayed a significant decrease ($P \leq 0.05$) in heterophil percentage compared to the heat-stressed

group. The silymarin group showed a significant reduction ($P \leq 0.05$) in heterophil percentage relative to the control group. A significant decline ($P \leq 0.05$) in basophil percentage was observed in the heat-stressed group compared to the control group. No significant differences ($P > 0.05$) were found in basophil percentage between the heat-stressed with silymarin group and the heat stress group. No significant alteration in basophils between the silymarin group and the control group. All experimental groups' eosinophil and monocyte percentages did not differ statistically.

Table 3. Role of silymarin in differential leukocyte count in male quails exposed to heat stress.

Treated	Lymphocyte %	Heterophil %	Eosinophil %	Basophil %	Monocyte %
Control group	39.25 ± 1.10 c	48.25 ± 1.75 b	3.75 ± 0.47 a	5.50 ± 0.28 a	3.25 ± 0.62 a
Heat stress 42°C group	32.75 ± 1.25 d	58.25 ± 1.65 a	3.50 ± 0.64 a	3.25 ± 0.62 b	2.00 ± 0.40 a
Heat stress 42°C with silymarin 200 mg/kg group	48.00 ± 0.91 b	43.75 ± 1.65 b	2.25 ± 0.25 a	3.50 ± 0.64 b	2.50 ± 0.64 a
Silymarin 200 mg/kg group	59.00 ± 3.71 a	31.50 ± 3.77 c	3.25 ± 0.47 a	4.00 ± 0.70 ab	2.25 ± 0.47 a

Different lowercase Letters in English in the identical column show a significant variance between the groups at a probability level of less than or equal to $P \leq 0.05$.

Table 4 demonstrates a notable rise in alanine aminotransferase enzyme levels in the heat stress group versus the normal control values. The data of the same table revealed a significant decline ($P \leq 0.05$) in the alanine aminotransferase enzyme in the heat stress with the silymarin group relative to the heat stress group. The silymarin group reported a significant decline in alanine aminotransferase enzyme in comparison to the control values. According to the present study's data, aspartate aminotransferase enzyme levels significantly increased ($P \leq 0.05$) in the heat stress group as compared to the control group. The results recorded a notable decrease in aspartate aminotransferase levels in the group with heat stress with silymarin compared to the heat stress. Table 4 didn't exhibit a remarkable difference in aspartate aminotransferase enzyme levels in the silymarin group. Table 4 illustrates a significant rise in alkaline phosphatase enzyme levels after heat stress

compared to the control values. The research data of group heat stress with silymarin showed a notable decrease in alkaline phosphatase enzyme compared to the heat stress group. There was no significant difference between the silymarin group and the control group. The results showed a significant decrease ($P \leq 0.05$) in liver and body weights in the heat stress group versus the control group. The present study results indicated no significant difference in liver and body weights in the heat stress with the silymarin group compared to the heat stress group. The results also showed no significant difference in liver and body weights in the silymarin group relative to the control group.

According to the current study's results, which are displayed in Table 5, there is no noticeable variation in total proteins, albumin, and globulin between all experimental groups.

Table 4. Role of silymarin on ALT, AST, ALP, liver and body weight in male quails exposed to heat stress. (Mean \pm SE):

Treated	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Liver weight g/100g B. W	Body weight/gm
Control group	42.8 \pm 3.66 b	259.1 \pm 12.46 b	114 \pm 11.8 b	1.68 \pm 0.10 a	199 \pm 6.73 a
Heat stress 42°Cgroup	83.6 \pm 23.80 a	316.6 \pm 22.03 a	191 \pm 20.2 a	1.30 \pm 0.09 b	173 \pm 7.40 b
Heat stress 42°C with silymarin 200 mg/kg group	38.8 \pm 5.59 b	250.3 \pm 7.04 b	108 \pm 14.90 b	1.56 \pm 0.08 ab	185 \pm 10.60 ab
Silymarin at 200 mg/kg group	33.0 \pm 2.91 c	226.3 \pm 10.60 b	117 \pm 5.20 b	1.65 \pm 0.04 a	198 \pm 4.16 a

Different lowercase Letters in English in the identical column show a significant variance between the groups at a probability level of less than or equal to $P \leq 0.05$.

Table 5. Role of silymarin on total proteins, albumin, and globulin of male quails exposed to heat stress. (Mean \pm SE):

Treated	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control group	3.60 \pm 0.12 ab	2.46 \pm 0.16 a	1.14 \pm 0.08 a
Heat stress 42°Cgroup	3.44 \pm 0.07 b	2.16 \pm 0.13 a	1.28 \pm 0.14 a
Heat stress at 42°C with silymarin 200 mg/kg group	3.78 \pm 0.29 ab	2.26 \pm 0.22 a	1.26 \pm 0.13 a
Silymarin at a dose of 200 mg/kg group	4.10 \pm 0.21 a	2.78 \pm 0.24 a	1.32 \pm 0.09 a

Different lowercase Letters in English in the identical column show a significant variance between the groups at a probability level of less than or equal to $P \leq 0.05$.

Table 6 illustrates that the heat stress group's cholesterol levels were noticeably greater than those of the control group. Additionally, there was no notable alteration in cholesterol in heat stress with the silymarin group compared to the heat stress group. According to the data presented in our study,

the cholesterol levels in the silymarin group and the control group did not differ greatly. The finding results demonstrate that triglyceride levels in the heat stress group were significantly greater than the control value. Our research indicates that heat stress with silymarin significantly lowers triglyceride

levels in comparison to the heat stress group. Based on the data in Table 6, there was certainly no statistically significant variance in levels of triglycerides within the silymarin and control groups. High-density lipoprotein levels dropped substantially in the heat stress group in comparison to the unaltered control group. The study found a substantial rise in high-density lipoprotein levels in the heat stress with the silymarin group relative to the group of heat stress. High-density lipoprotein levels

improved significantly in the silymarin group linked to the control values. The current research found a significant rise in HSP 70 in the group of heat stress associated with the control group. The study discovered a substantial reduction in HSP 70 in the heat stress with the silymarin group as opposed to the heat stress group. The silymarin group showed no significant alteration in HSP 70 compared with the control value.

Table 6. Role of silymarin on cholesterol, triglyceride, high-density lipoprotein and heat shock protein 70 levels in male quails exposed to heat stress (Mean \pm SE):

Treated	Cholesterol (mg/dl)	Triglyceride (mg/dl)	High density lipoprotein(mg/dl)	Heat shock protein 70(ng/ml)
Control group	207 \pm 10.83 b	151 \pm 37.37 b	155 \pm 5.03 b	2.99 \pm 0.92 c
Heat stress 42°Cgroup	251 \pm 4.68 a	271 \pm 15.13 a	138 \pm 9.59 c	5.11 \pm 0.18 a
Heat stress at 42°C with silymarin 200 mg/kg group	227 \pm 16.98 ab	224 \pm 21.49 b	149 \pm 13.05 b	4.13 \pm 0.20 b
Silymarin 200 mg/kg group	201 \pm 16.01 b	176 \pm 18.38 b	182 \pm 14.09 a	3.27 \pm 0.26 c

Different lowercase Letters in English in the identical column show a significant variance between the groups at a probability level of less than or equal to $P \leq 0.05$.

DISCUSSION

According to the current study's hematological findings, heat stress caused severe anemia represented by a decrease in RBC count, PCV%, Hb concentration, MCV, MCH, and MCHC. This result was consistent with a similar study that showed that exposure of broiler chicks to heat stress caused differences in erythrocyte number, Hb, MCV, MCH, and MCHC [16].

Our result also matches another result that showed a reduction in the Hb and PCV% when exposed to heat stress in broiler chickens

[17]. The etiology of our findings could be explained by the fact that because the erythrocytes contain high oxygen levels, the RBCs are extremely vulnerable to oxidative stress. Furthermore, the erythrocytes are considered to represent the principal cells susceptible to oxidative injury [21], or exposure to heat stress related to the instability of various biochemical compounds [22], including the erythropoietin hormone synthesis. Erythropoietin is a hormone secreted in the kidneys that stimulates the generation of pro-erythroblasts from

hematopoietic cells in the bone marrow. It is a major hormone that regulates the process of producing, differentiating, developing, regulating, and promoting red blood cells [23].

Elevated temperature stress in chickens inhibited the formation of erythrocytes in the bone marrow. High temperature causes hemolysis and drops in hematocrit and hemoglobin levels. Also, elevated temperatures impact hemoglobin molecule construction of disulfide cross-bonds between hemoglobin chains and distort the hemoglobin molecule, which results in the formation of Heinz bodies [24].

finally, all the reasons mentioned above led to a decrease in MCV, MCH, and MCHC. The current study recorded that heat stress led to a decrease in WBC count; a similar study confirmed that high temperature triggered a decline in the number of WBCs in broilers [25]. That gradually diluted blood cell concentrations, particularly white blood cells, so one of the reasons for the decrease in white blood cells is the increased glucocorticoid hormone levels in plasma under acute heat stress, which produced leucopenia. [26], the occurrence of leucopenia as the effect of high temperature exposure was also reported by [27] due to the close relationship between leukocyte profile and glucocorticoid hormone level in the blood.

The increased secretion of glucocorticoid during heat stress exposure could reduce granulocyte and lymphocyte cell counts and heterophil percentage. This phenomenon occurs in all vertebrates, both in natural stress conditions and artificially stressed conditions, by glucocorticoid hormone sublimation. The current study recorded that exposure to heat stress with the silymarin group caused an increase in Hb and no changes in PCV.

This result matched with Fathi et al. [17]. Also this result agrees with Abo Shahema [28],

who recorded an increase in Hb and no change in PCV% in developed chickens with silymarin supplementation during the summer season, and our current study recorded a significant increase in RBCs, MCV, and MCH, as well as MCHC, in heat stress with the silymarin group, and this study disagrees with a study that found that silymarin didn't cause a difference in RBCs, MCV, MCH, and MCHC in laying hens under summer conditions [28]. The present study shows a high WBC count in heat stress with the silymarin group; this result is similar to a prior study [28].

Additionally, our outcome corresponds with Talebi [29], who mentions that silymarin increases the WBC count of chickens suffering from high environmental temperatures; this may be due to the role of silymarin in high-temperature circumstances in improving the immune status of the stressed birds [10]. The current findings revealed that there was no difference in RBCs, PCV, and Hb in the silymarin group. These results were similar to the results of another investigation, which showed that silymarin didn't cause a difference in the data of RBCs, PCV, and Hb content in Japanese quails [30]. The present research findings demonstrated that silymarin treatment increases WBC count. This finding was in agreement with a prior study's findings that silymarin increases WBCs in broiler chickens at varying dosages [31]. To explain this rise in leucocytes, silymarin improves immune system functions in the body [32].

In our current study, the results show a decrease in lymphocytes and a rise in heterophils and basophils and the heterophil/lymphocyte proportion in the heat stress group. This finding agrees with a finding of a study that recorded that exposure to heat stress caused difference in DLC of broilers [16]. To explain these changes, heat stress has been associated with decreased leucocyte response due to the effect of circulating ACTH hormone on corticosterone levels and altered

circulating leucocyte components [33]. Stress due to heat raised the H/L ratio. This result is due to physiological stress [34].

The present study recorded that group heat stress with silymarin caused a rise in lymphocyte and heteropenia and heterophil/lymphocyte proportion; for the majority of stressors, the heterophil/lymphocyte ratio can be utilized to identify the existence of physiological stress. On the other hand, extreme stress can cause heteropenia [35]. These results were in line with a previous study that recorded an increase in lymphocytes and a decrease in heterophils and the H/L ratio of laying hens in the hot season [28], and these results indicate that silymarin improved the immunity in high temperatures due to the action of silymarin as an antioxidant, scavenging ROS and reducing lipid peroxidation. [36]. The result of the present study shows that the silymarin group displays a rise in lymphocytes and a decline in heterophils and stress index. This study disagrees with a previous study finding that silymarin didn't cause a difference in lymphocytes, heterophils, and heterophil/lymphocyte proportion in broilers [37].

The data of the current results revealed an increase in the levels of liver enzyme functions and a decrease in liver and body weight in quails when exposed to high temperature. This outcome was in line with the findings of other researchers who found an increase in liver enzymes in broiler chickens exposed to heat stress [38]; a similar result shows an increase in AST in broilers exposed to heat stress [39]. The reason for this increase in ALT and AST is related to liver injury [39]; high ambient temperature can cause oxidative damage to a variety of livestock species, including broilers [40]. Furthermore, oxidative stress damages biomacromolecules like DNA, proteins, and lipids, causing cell dysfunction and even tissue destruction [41], and the result

about liver and body weight correlated with the findings of a previous study conducted on broiler chickens [16]. The explanation for the decline in liver and body weight could be ascribed to the birds' lower feed consumption and growth, as heat stress impairs growth performance [42]; also, excessive heat inhibited liver function and caused damage to liver cells in broilers [39]. Furthermore, induce higher breakdown of cells and less building of cells in the body, resulting in weight loss [43]. The present investigation appeared that the group with heat stress with silymarin caused a decrease in ALT and AST; these results were the same as with [28, 17].

On chickens, which showed a decrease in ALT and AST when silymarin was used under summer conditions. One important indicator of liver function is the level of activity of the enzymes AST and ALT, which catalyze the transfer of amino groups across amino acids. Changes in the transamination and metabolic restructuring processes of broiler chicks fed milk thistle extract may be the cause of the observed fluctuations in the content of AST in their blood serum [31]. To explain this decline in liver enzymes, silymarin improved hepatic damage in chickens [43]. One well-known potent antioxidant and toxin reducer is silymarin, which helps minimize the effects of stress [44], and by blocking the enzymes that produce free radicals and activating a set of antioxidants that minimize inflammatory effects, silymarin acts as an antioxidant to prevent the generation of free radicals [45]. Results of our current research showed a decrease in ALP in heat stress with the silymarin group; it contradicted the findings of that, which found no noticeable differences in ALP level [28]. The current studies on quails demonstrated that silymarin decreases ALT in the birds. This outcome was in line with a prior investigation on ducks that found a notable drop in the ALT level [46]. ALT was considerably reduced, which

indicates silymarin enhances liver function and has no negative effects on liver enzyme functions [47, 48].

It states that silymarin promotes protein and nucleic acid production, which helps with repair and regeneration of liver tissue. According to the current study's findings, silymarin showed no discernible effect on AST, ALP levels, liver, and body weight; these outcomes were in line with a study that found silymarin did not alter AST, ALP, liver, and body weight in broilers [49]. The current study's observations demonstrated that all groups of the experiment had the same levels of albumin, globulin, and total proteins. This result was consistent with another researcher [50, 51]. The results of our research indicated that heat stress causes an increase in cholesterol and triglycerides and a decrease in high-density lipoprotein (HDL). This finding is in harmony with [52].

To explain this increase in lipid profile, heat stress causes overactivity in adrenal glands, which generate corticosteroid hormones that may be the reason for this rise in cholesterol levels [53]. Also, because of several metabolic pathways implicated in raised lipid mobilization and catabolism in quails [54], the result about HDL in our study lines up with [55], which demonstrated heat stress negatively affects HDL levels in quails. Our study results indicated that the group with heat stress with silymarin led to a decline in cholesterol and triglycerides and a rise in HDL levels. This result was similar to [28, 17]. One possible explanation for changes in lipid profile could be silymarin's hypolipidemic effect and protective effect on LDL peroxidation [56].

Furthermore, [57] found that silymarin is a potent antioxidant that has been shown to promote liver cell regeneration and reduce blood cholesterol. Silymarin could be a new agent in the prevention and treatment of

hypercholesterolemia and atherosclerosis [58]. The silymarin group in the present study showed an increase in high-density lipoproteins, and these results are linked with another study on ducks, which also showed an increase in the level of high-density lipoproteins. [59]. Silymarin has a synergetic effect that has a preventative function in protecting from atherosclerosis through improvement of HDL [47]. The results of our study indicated that heat stress increases HSP70. These results line up with [16], who discovered that heat stress exposure raises HSP70 in broilers. The elevated HSP70 level could be attributed to increased HSP70 gene expression in liver cells during temperature changes [60].

The HSP70 gene is extremely sensitive to high temperature [61], and raised levels of HSP70 are a key protective factor against negative alterations in protein structure and function caused by various stressors [62]. Our present study recorded that heat stress with silymarin shows a decline in HSP70. This result lines up with the result indicating a decrease in gene expression of HSP70 in serum [63]. This decline in the level of HSP70 is due to the action of silymarin as a potent antioxidant [2].

CONCLUSION

Silymarin minimized the harmful effects of heat stress on physiological parameters such as blood characteristic, liver functions, and heat shock protein 70 in male quails.

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CONFLICT OF INTEREST

No conflicts of interest have been disclosed by the researchers

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