HISTOLOGICAL AND MOLECULAR STUDY OF SPLEEN IN *Japanese Quail* UNDER THERMAL CONDITION

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ABSTRACT

The present study was conducted to determine the histological and Molecular changes in the spleen of adult male *Japanese quail* after being exposed to effect of thermal stress was recorded. This study included two groups, each group consisting twelve male birds, the control group **(A)** was exposed to normal temperature for 45days, while the group (**B)** exposed to temperature (42^{O}C) for 45 days. The histological and molecular changes were studied during (15, 30 and 45) days of the experiment, histological changes in spleen were represented by necrosis, degeneration hemorrhage and shrinkage fibrosis of lymphocytic nodules, cytoplasmic vaculation, infiltration of lymphocytes, metamorphosis of lymphocyte, nuclei pyknotic, dilation in the central vein of lymphatic nodules, edema, dilation of sinusoids and congested blood vessels. Molecular examination showed that the heat shock protein (hsp70) gen is present in temperature group that is also found in the control group. This confirms that the hsp70 gen is present in birds at normal and abnormal conditions.

INTRODUCTION

Japanese quail is a small bird that has a fast growth process, it is a species or subspecies to the genus *COTURNIX*. These birds are called by many names such as: common quail, Japanese Gray quail, Japanese Migratory quail, king quail and Japanese king quail. Coturnix: is a term used to refer to this Japanese quail (1, 2 and 3). High ambient temperature and humidity are the major stress factors affecting the

birds during summer (6). Stress is the non-specific response of the body to any request while the stressors may be defines as a factor that provides stress at any time (7). Heat stress has a negative balance between the net amount of energy that formed or resulted from animal's body to it surrounding environment and the amount of heat energy that is producing by animal (8, 9 and 10). Many types of birds are responding similarly to heat stress expressing some individual changes in intensity and period of their response (11). In response to heat stress, there is a protein produced by cells that is exposed to stressful conditions called Heat shock protein (HSP). These proteins contained a low molecular weight. HSP have specific functions on cell growth and in preventing damage caused by stress (12).

The effect of high environmental temperature on histology structure of some internal organs demonstrated that the high temperature caused pathological changes (6). While chronic heat stress has negative effects on the performance and

physiological characteristics of poultry, heat stress also effects on immune response. (13) Proved that the development of the specific immune response of young chickens was affected after exposed to temperatures from (44.4^{o}C - 47.8^{o}C). The decrease of white blood cells and the increase in the heterophil/lymphocyte ratio, as indicator for heat stress which has effect of the immune response of bird (14, 15and 16). The aim of the present study was to determine the effect of the thermal stress on histological and molecular changes of a male *Japanese quail* birds.

MATERIALS AND METHODS

A total of 24 males of *J.Quail* were purchased from the local market in Basrah province within body weight average (162–172). The birds were reared in separated cages at college of veterinary medicine in basrah university, and the birds were divided into two groupe:

Control group (A): contains twelve quails exposed to normal temperature.

Group B: contains twelve quails exposed to high temperature (42).

All birds were killed and the spleen was isolated and kept in 10% of buffered neutral formalin solution immediately after removal. After fixation for 72hr., the specimens were washed with running water for 2hr, then after that dehydration was done with alcohols concentration from 70%, 80%, 90% and 100% for 2hrs to each concentration, then clearance was done by xylol, after that the specimens were embedded with paraffin wax and sectioned by microtome at 5µm for tissue. After that all the sections were stained with haematoxylin and eosin stain (H&E) (17).

The blood samples were collected directly from the heart of all quails at days 45th, the blood samples were transferred into anti-coagulant tubes that contain EDTA-K3 and used to molecular examination.

mRNA Extraction: First strand mRNA extraction by using SV Total RNA Isolation System kit.

cDNA Isolation

Genomic cDNA was isolated from *J. Quail* by RT/PCR Premix kit.

PCR Coding Gene

After isolating the cDNA from *J. Quail*, it is used as a template for PCR according to typical conditions of PCR amplification.

Agarose Gel Electrophoresis

Electrophoresis was used according to typical conditions and read by a UV transilluminator.

RESULT AND DISCUSSION

Histological Study:

The examining sections of control group spleens showed the normal size and shape of blood vessels, lymphocyte and other structures, Figure (1)

While the treated groupe revealed histological changes in splenic section of birds exposed to thermal stress (42^{o}C) for (15, 30 and 45) days were represented by necrosis and degeneration of lymphatic nodules, lymphocytes infiltration, hemorrhage and congested of lymphatic nodules, dilation of sinusoids, hemorrhage

of both red and white pulp tissue, dilated and congested of the blood vessels, degeneration of lymphocyte, hemosiderin as in figure (2 and 3). The present study was agree with (18) who reported that the exposure to heat stress appeared of noticeable pathological changes among splenic parenchyma compared with control, These changes include lymphocytic necrosis and degeneration especially at the lymphoid nodules periphery, also lymphocyte infiltration. Additionally, massive congested areas within the splenic red pulp, congested blood vessels and hemosiderosis were noted. (19) Also showed that the histological changes were characterized by severe vascular congestion of red pulp and atrophy of peri-arteriolar lymphoid tissue in white pulp. (20) reported that the heat stress included congested blood vessels and sinusoids among the red pulp, also the hemosiderosis was observed as yellowish brown pigment inside the phagocytic cells. Moderate to severe lymphocytic depletion was represented by smaller lymphatic nodules, also degeneration and necrosis of lymphocytes especially at the peripheral of lymphoid follicles. While (6) reported that spleen did not show any pathological changes in all the varieties. The results of cDNA after running the PCR product in the gel, the band of PCR product (HSP70 gene) prepared from RNA then converted to cDNA appeared ≅ 960 bp. This indicates that the exposure to temperature (42^{o}C) caused stimulation the production of heat shock protein gen to protect the birds. The result recorded that in each of normal and abnormal condition was caused production of Hsp70 gene. These results coincided with (21) who reported that young animals were capable of inducing Hsp70 protein in the early phases of

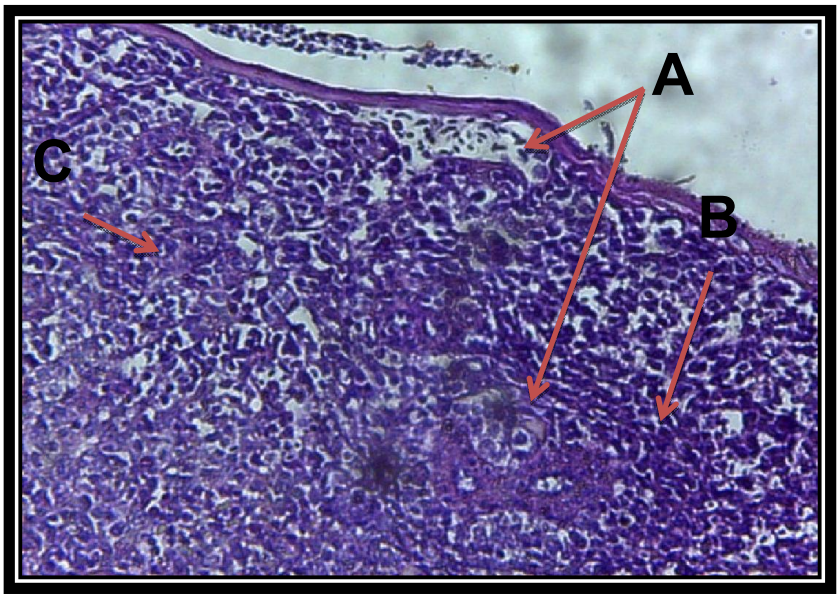

Figure 1: Transverse Spleen Section of control group shows normal tissue structure (A) White pulp, (B) Red pulp, (C) Lymphatic nodules **(H&E 400X)**

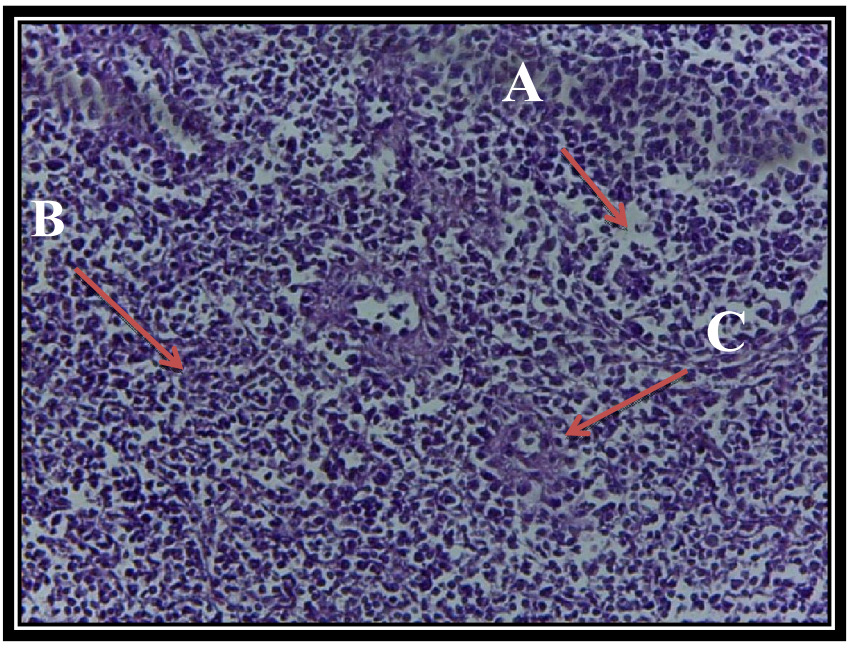

Figure 2: Transverse Spleen Section after exposure to 42^{O}C for 15 days shows (A) necrosis and disintegration of lymphatic nodules, (B) lymphocytes infiltration, (C) increased density of splenic cord **(H&E 400X)**

recovery 1 hr after a heat challenge. However, at 1 hr and other time periods of recovery, old animals failed to maintain the high Hsp70 protein levels. (220 and (23) indicate young animals groups were capable of up-regulating Hsp70 level at (24 & 72) hr after of heat stress. Also (21) reported that induction of heat shock proteins including HSP70 that gives a cytoprotective effect against further stress.

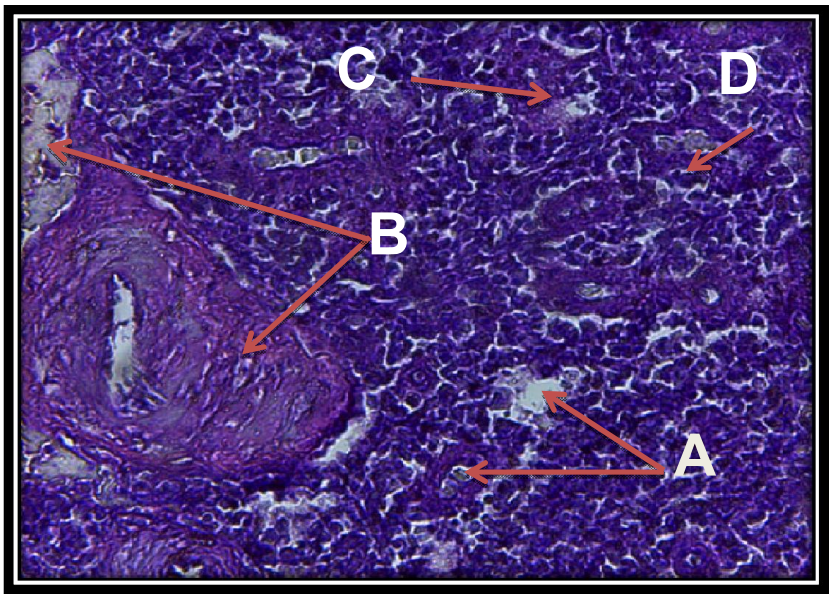

Figure 3: Transverse Spleen Section after exposure to 42^{O}C for 45 days shows (A) necrosis and degeneration of lymphatic nodules,(B) hemorrhage and hyperplasia of vessel wall and edema, (C) cytoplasmic vaculation, (D) disappeared some of lymphatic nodules **(H&E 400X)**

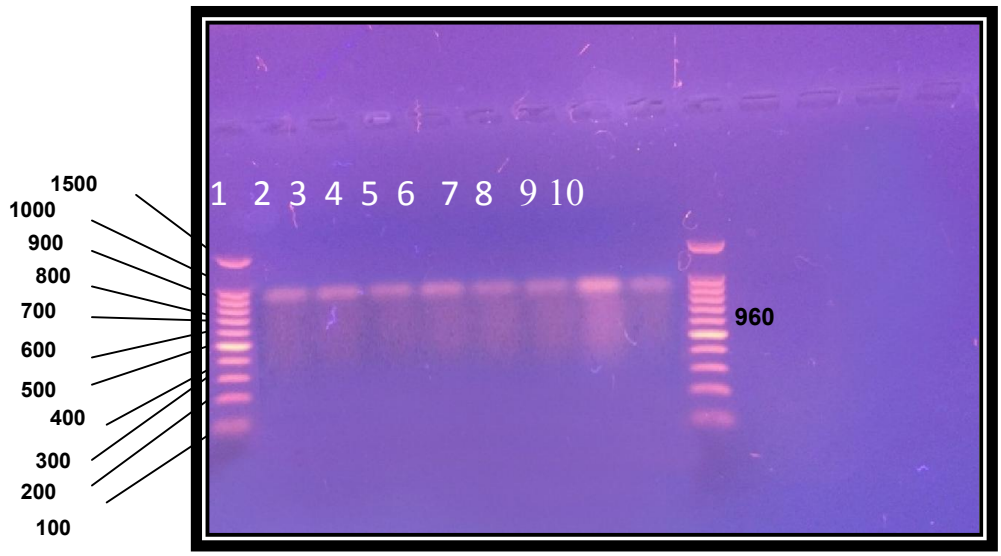

Figure 4: Electrophoresis Gel of PCR product of isolated RNA, lane (1, 10) the ladder, lane (2, 3, 4, 5) the band from the cDNA (control), lanes (6, 7, 8, 9) the band from the cDNA (temperature 42°C)

دراسة نسيجية وجزيئية للطحال في طيور السمان تحت تأثير ألإجهاد ألحراري

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الخلاصة

أجريت هذه الدراسة لمعرفة مدى تأثير الاجهاد الحراري على المستوى النسيجي والجزيئي في الطحال لذكور طيور السمان البالغة. قسمت الطيور الى مجموعتين تضمنت كل مجموعه (12) حيوان وكالاتي: مجموعة السيطرة عرضت الى درجة حرارة الجو بينما المجموعة الاخرى عرضت على مدار اليوم لدرجة حرارة (42م) ولمدة 45 يوم تم خلالها دراسة التغيرات النسيجية والجزيئية خلال (15-30-45) يوم من مدة التجربة. اظهر الفحص النسيجي وجود تغيرات في الطحال مثل تنخر و تنكس ونزف وانكماش وتليف العقد اللمفاوية والتفجي السايتوبلازمي وارتشاح وتغيير شكل الخلايا اللمفاوية وتغلظ الانوية وكذلك لوحظ توسع في الوريد المركزي للعقد اللمفاوية وتجمع الخزب وتوسع الجيوب اللمفاوية واحتقان في الاوعية الدموية. بينما اظهرت نتائج الفحص الجزيئي وجود جين مسؤول عن انتاج بروتينات الصدمة الحرارية في

مجموعة الحرارة(42°C) كما وجد في مجموعة السيطرة مما يؤكد ان هذا البروتين يوجد في كل الظروف الطبيعية والغير طبيعية للحيوان.

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