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Histological and Molecular Study of Bone Marrow 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 bone marrow 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 bone revealed hemorrhage, degeneration and hyperpigmentation of hemopoietic tissue, seperation of hemopoietic layer from endosteum, necrosis, degeneration of periosteum, degeneration and disappeared of adipose tissue and hemopoietic tissue. 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 condition.

Keywords

Hemorrhage, hyperpigmentation, necrosis, hsp70 gen

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1-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, 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, 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, 15, 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.

2-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 bone was isolated, because of bone hardness, it must undergo decalcification by putting it in diluted nitric acid solution 5% for 1 day, and this solution dissolved all these calcareous salt. It becomes simple to cutting. This tissue 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.

3-Result

Histological Study:

The examining sections of control cancellous bones showed normal shape and size of osteocyte, bone marrow, adipose tissue, lacuna and periosteum , Figure (1)

While the treated groupe revealed histological changes in cancellous bones section of birds exposed to thermal stress (42^{o}C) for (15, 30 and 45) days were represented by hemorrhage, degeneration and hyperpigmentation of hemopoietic tissue, seperation of hemopoietic layer from endosteum, necrosis and degeneration of periosteum, degeneration and disappeared of adipose tissue and hemopoietic tissue, cytoplasmic vaculation, analysis of osteocytes, degeneration, necrosis and fragmentation of bone marrow figure (2 and 3).

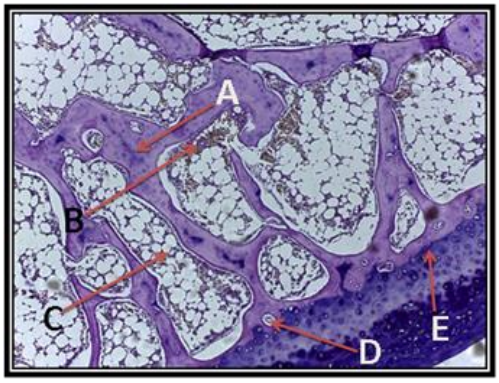

Fig. 1- Transverse section in the cancellous bones of control birds shows a normal histological structure (A) osteocyte (B) bone marrow (C) adipose tissue (D) lacuna (E) periosteum (H&E 400X)

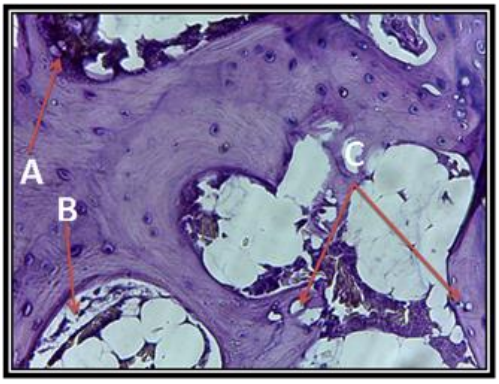

Fig. 2- Transverse section in the cancellous bones after exposure to 42^{O}C for 30days shows (A) hyperpigmentation of hemopoietic,(B)degeneration in some of hemopoietic tissue, (C) degeneration and necrosis of bone trabecula (H&E 400X)

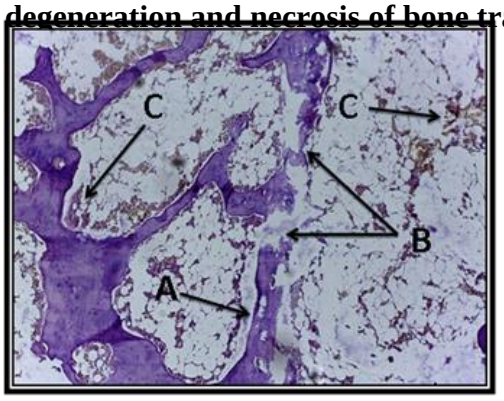

Fig. 3- Transverse section in the cancellous bones after exposure to 42^{O}C for 45days shows (A)sepreation of endosteum, (B) antolysis of endosteum of bone trabecula (C) degeneration some of marrow tissue. (H&E 400X)

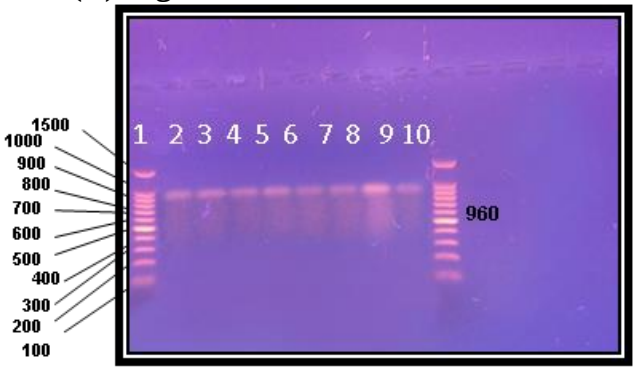

Fig. 4- Electrophoresis Gel of PCR product of isolated RNA, lane (1, 10) the ladder, lane (2, 3, 4, 5) the band from the cDNA (control), lanes (6, 7, 8, 9) the band from the cDNA (temperature 42ºC)

4-Discussion

The results are in agreement with [4] who reported that the bone is more susceptible to thermal injury in the rabbit.[5] reported that the heat stress at 50℃ for 1 min or 47℃ for more than 1 min prevent osteoblast degeneration and causes bone resorption and conversion to adipocytes, thus leading to failure of osseous tissue formation in the rabbit. also reported that thermally induced bony change is not an immediate occurrence but a slow-developing process that extends over a period of 4 weeks.[18] confirmed that the temperature could increase above 47^{o}C and cause irreversible osteonecrosis.[19] reported that bone necrosis was consistently seen in histologic sections at scald temperatures greater than or equal to 70^{o}C, damaged periosteum and showed evidence of either bone or marrow necrosis.[20] showed that along with the temperature increase, the area of dead osteocytes increased and degeneration of the periosteal membrane was delayed. Also [21] found that thermal damage leading to death of native bone cells (osteocytes, osteoblasts, osteoclasts and mesenchymal stem cells. 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 [22] who reported that young animals were capable of inducing Hsp70 protein in the early phases of 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. [23, 24] indicate young animals groups were capable of up-regulating Hsp70 level at (24 and 72) hr after of heat stress. Also [22] reported that induction of heat shock proteins including HSP70 that gives a cytoprotective effect against further stress. In conclusion ?

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