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Ali S Al-Hassani
Lecturer. Agriculture College, Baghdad University, Iraq

Correspondence
Ali S Al-Hassani
Lecturer. Agriculture College, Baghdad University, Iraq

Histological effects of growth hormone (GH) and Insulin-like growth factor-1 (IGF-1) on heart, liner and gizzard in chickens

Ali S Al-Hassani

Abstract
In the present study, histological study was carried out on adult chickens to focus the light on the effects of GH and IGF-1 on heart, liver and gizzard. The microscopic examination had shown that GH and IGF-1 promotes protein synthesis in the heart tissue. The herein work referred to the presence of a considerable amount of adipose tissue among the bundles of cardiac muscles which is related to the metabolic process. The results also revealed that GH and IGF-1 promotes both protein synthesis and Mitosis in the tissues of liver and gizzard. Moreover, the above hormones stimulate apoptosis, regeneration, and secretory activity in gizzard secretory glands.

Keywords: Histology, chicken, GH, IGF-1, internal organs

1. Introduction
Many cell types are responsible for producing IGF-I which can plays both endocrine and paracrine/autocrine functions [17]. The major source of IGF-I is the liver as a circulating endocrine growth factor which is regulated by growth hormone [4]. The importance of IGF-I for the growth of cells has been confirmed both in vitro and in vivo. It has been shown that mice with complete disruption of IGF-I gene expression exhibit marked in utero and postnatal growth retardation [10]. The protective effect of IGF-I on cell survival has been known for several years, in particular in the central nervous system [6]. IGF-I can also play a role in controlling differentiation of certain cell types. For example, under certain conditions, myoblasts, osteoblasts, adipocytes, oligodendrocytes, neurons, and hematopoietic cells can be induced to differentiate by IGF-I [12]. Like many other protein hormones, GH considered as an anabolic for the tissues by attaching specific receptors on the cells [4]. The effect of (GH) and (IGF) on many internal organs in the body has been studied by many researchers who reported that GH inhibits the process of apoptosis [3, 8]. Because of its nature as polypeptides and not as fat-soluble, it need a receptor on targeted cells to penetrate the fatty plasma membrane [2]. The liver is the target organ of GH and the principal of IGF-1 production which affect the growth of many types of tissue. GH serves many functions; It raises the concentration of fatty acids [14] used as anti-aging agents [9] and maintaining muscle mass [5]. GH is still considered as a very complex hormone, while many of it's function remain unknown [13]. Therefore the objective of this study is to explore the function of IGF-I and GH and the effects of the two hormones on the heart, liver and gizzard in broiler.

2. Materials and Methods
This study was carried out in one of broiler farms. Three hundered birds of two commercial broiler chickens (Cobb500 and Hubbard F-15) with both sex (male and female) were raised from 1st day until 7th weeks of age. All chicks were fed a starter diet in mash form containing 23.6% crude protein and 3026 kcal ME/kg during first 10 days of age, then grower diet was fed containing 21.6% crude protein, and 3146 kcal ME/kg during 11-24 days of age and finally finisher diet was fed containing 20% crude protein, and 3197 kcal ME/kg during 25-49 days. The three diets were formulated according to NRC (1994). Samples of hearts, livers and gizzards from adult chickens with different concentration of GH & IGF-1-in serum were obtained and prepared (Ten for each). Samples were fixed in 10% neutral buffered formalin for 48 hrs, and then specimens were dehydrated with ethyl alcohol in increasing concentrations (70% to 100%), embedded in paraffin, sectioned at 5-7 um and stained by Hematoxylin and Eosin [12]. High magnification was used for inspection through light microscope [6].

3. Results and Discussion

3.1 Heart

Current study revealed that the cardiac muscle cells in atrium of chickens with different concentration of GH & IGF-1 were well formed, and had a highly eosinophilic cytoplasm. The mostly oval nuclei were euchromatic (light staining) with numerous prominent nucleoli (Fig.1). The eosinophilic appearance may be due to the presence of cationic components such as proteins with many ionized groups that have the affinity for acidic dyes [12]. The number of mitochondria is related to the cell's energy needs. Thus, cells with high-energy metabolism (i.e. cardiac muscle) have abundant mitochondria which give the eosinophilic appearance to the cytoplasm [11]. Mitochondria were often large enough to be visible with light microscope as numerous discrete eosinophilic organelles [1]. New mitochondria originate from pre-existing mitochondria by growth and subsequent binary fission of the organelle itself. The blood supply of the cardiac muscle cells was increased. Adipose tissue was abundant and interposed between the bundles of myocardium. Darkly stained heterochromatic nuclei were not recorded, as the cardiac muscle has no regenerative capability beyond early childhood [15]. The intensity of nuclear staining of the chromatin is frequently used to distinguish and identify different tissues and cell types under light microscope. Generally, cells with lightly stained nuclei are more active in protein synthesis than those with condensed dark nuclei. In light stained nuclei with much euchromatic and few heterochromatic clumps, more DNA surface is available for the transcription of RNA. On the other hand, the current study suggested that purkinje fibers were clearly apparent and easily distinguishable from the contractile muscle fibers due to sparsity of myofibrils [18]. These cells were present in the sub endocardial layer. They were larger than the cardiac contractile cells. Large amounts of lightly stained glycogen filling the center of these cells around the nucleus leaving a thin band of myofibrils at the periphery. The cells had abundant cytoplasm, large nuclei, and prominent and numerous nucleoli (Fig.2). All of which indicate extensive protein synthesis to maintain the efficient function of the cardiac muscle cells [11]. Ganong [4] reported that GH secretion declines in old ages, and there has been considerable interest in injecting GH to counterbalance the effect of aging. The current results referred to the presence of a close relationship between fibroblast and cardiac muscle cell (Fig. 1) and the presence of the active fibroblast among the bundles of cardiac muscle, which appeared as large with euchromatic nuclei and basophilic cytoplasm. Mesher [11] stated that various growth factors (present in fibroblasts) influences cell growth and differentiation. The herein work referred also to the presence of a considerable amount of adipose tissue among the bundles of cardiac muscles which is related to the metabolic process. That is to say, the numerous mitochondria including the appropriate enzymes that allow fatty acids to be oxidized [15].

3.2 Liver

The current study found that the liver of chickens with different concentration of GH & IGF-1- in serum was highly irrigated with nucleated blood of the poultry sinusoidal capillaries. Hepatocytes present abundant basophilic cytoplasm and large, ovoid, pale nuclei containing fine chromatin and one or more prominent nucleoli. Dark nuclei, binucleated hepatocytes were also shown suggesting the presence of different stages of mitosis (liver hyperplasia). The sinusoidal capillaries between the hepatic plates were highly engorged with nucleated erythrocytes **(**Fig. 3, 4). The basophilic cytoplasm may be attributed to the rich rough endoplasm reticulum and well developed Golgi complex while the pale-staining cytoplasm results partly from the diffuse nature of the granular endoplasmic reticulum [1]. GH stimulates the liver to produce (IGF-1) [2] which in turn enhances the multiplication or development various types of cells by affecting the metabolism of proteins, carbohydrates and fats. Growth factors are produced by macrophages and lymphocytes and are important in regulation of the immune system [4]. Scopa *et al*., [16] stated that the treatment with GH & IGF-1 in rats with experimental obstructive jaundice, improves liver histology especially mucosal DNA. The herein study was in variance with many researchers and with Bogazzi *et al*. [3] who stated that growth hormone inhibits apoptosis. In conclusion, the current study revealed that growth hormones trigger apoptosis for eliminating unwanted cells and promotes mitosis for tissue building.

3.3 Gizzard

Two forms of gizzard follicles were recognized, the most numerous were filled or partly- filled follicles; Other empty follicles were also recognized**.** Exfoliated apoptotic follicular cells were bulged then exfoliated to the lumen of follicles (Fig.5). The study regarded the former as stimulating stage, and the latter as resting stage. This typing was reverse to the follicles of thyroid gland [7, 17]. The herein work found that the glands of the gizzard consisted of three types of well-formed cells; the first type was the basal cells that lie in the fundus of the gland. They were very few, cuboidal in shape with pale cytoplasm and round pale nuclei containing single prominent nucleolus. The second type was the chief cells that lies at the base of the gland. They were numerous in number, cuboidal to columnar in shape with strongly basophilic cytoplasm and rounded indented nuclei. The basophilic appearance of cytoplasm may be due to the rough endoplasmic and numerous free ribosomes. Flattened cells with oval nuclei may be detected; the third type was the surface cells that lining the pit of gland; they were taller than the chief cells. Their nuclei were irregular and dark. Chief & surface cells had dark heterochromatic nuclei that liable for mitosis. The most striking result of the current study was capability of (GH) &IGF-1 to induce the mechanism of apoptosis and thereafter the regenerative capability of the glandular secretory cells (Fig.6).This finding was in line with result obtained by Kolle *et al*., [8] who reported that GH promotes cell proliferation and inhibits apoptosis. In conclusion, the present study revealed that (GH) & (IGF-1) promote the regenerative capacity of the glandular tissue.

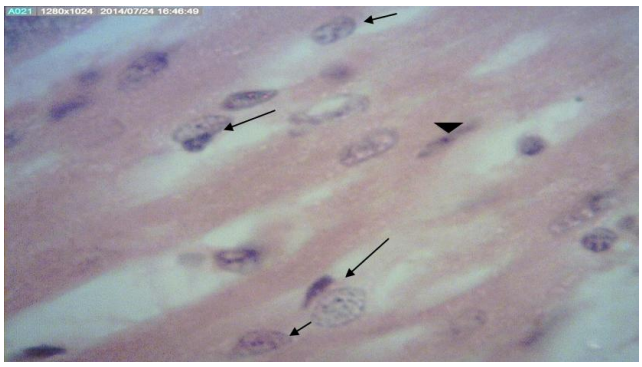

Fig 1: Heart atrium of chicken. Note the attachment and relation between the nuclei of fibroblast and cardiac muscle (long arrows), eosinophilic myocardium, euchromatic pale nuclei and thier numerous prominents nucleoli of cardiac muscle cell (short arrows). and the spindle-shaped fibroblast with prominent single nucleolus (arrow head). X 1000. H&E stain

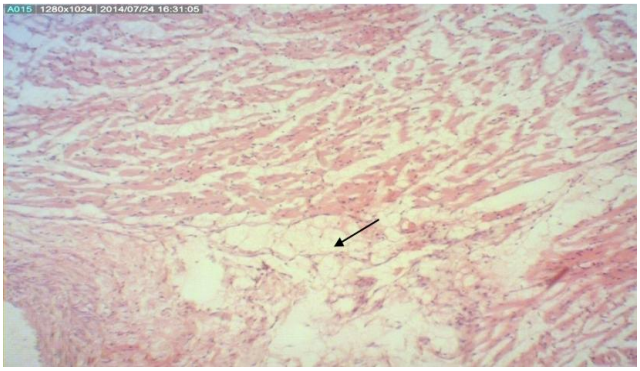

Fig 2: Transverse section in heart atrium of adult chicken nourished with (GH) & (IGH-1). The upper myocardium and the lower prominent purkinje fibers (arrow). X100. H&E stain

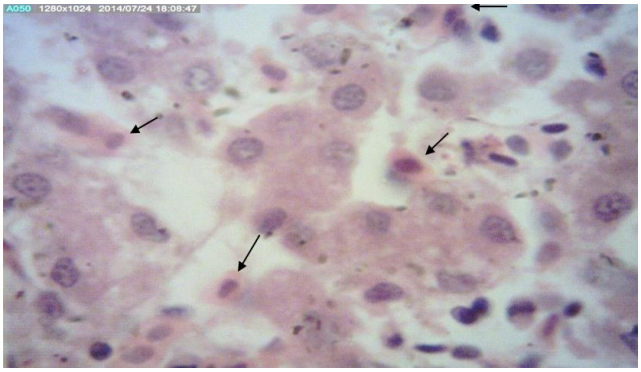

Fig 3: Chicken liver nourished with (GH) & (IGH-1) –loaded diet. Note the hepatocytes with euchromatic nuclei. The arrows refer to the nucleated red blood cells of the sinusoidal capillaries.X1000. H&E stain

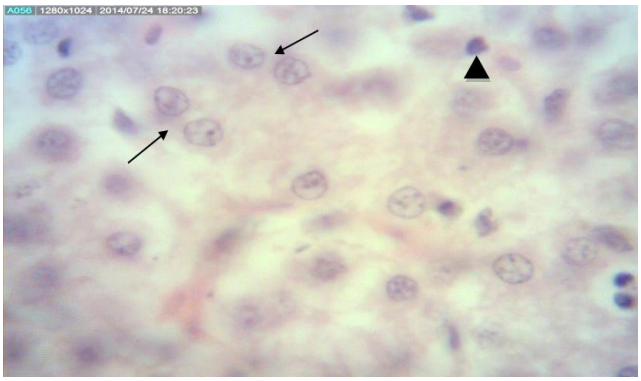

Fig 4: Liver of chicken nourished with (GH) & (IGH-1) -loaded diet. Euchromatic nuclei with numerous prominent nucleoli; Binucleated cells (arrows). Dark apoptotic nuclei (pyknosis) were present (arrow head). X1000. H&E stain

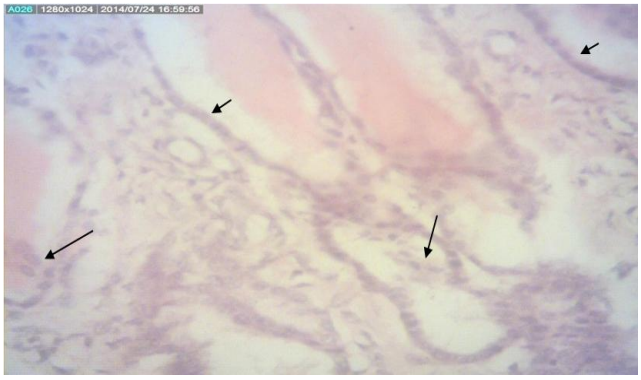

Fig 5: Secretory follicles of gizzard in different functional stages lined mostly with chief cells (short arrows). Two bulging surface cells were exfoliated (long arrows). Exfoliated apoptotic cells found in the lumen of the follicles.X400.H&E stain

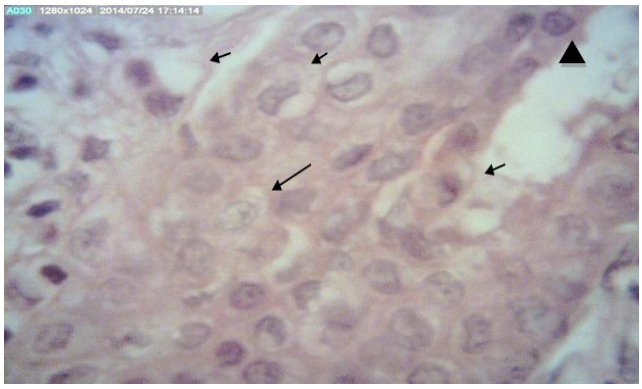

Fig 6: Secretory glands of chicken gizzard. The most numerous chief cells (arrow head). Small arrows pointed to the surface cell in the pit. Large arrows referred to the basal cell in the fundus of the gland. X1000.H & E stain

4. Conclusion

The most striking result of the current study was the capability of (GH) &IGF-1 to induce the mechanism of apoptosis and thereafter the regenerative capability of the glandular secretory cells.

5. Acknowledgement

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