Histomorphological developmental study of the adrenal gland of the local rabbit at one and fifteen days age

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Abstract

The adrenal glands are complex endocrine glands regulating multiple physiological processes in the animal body. This study was carried out on the adrenal glands during two postnatal periods of local rabbit. Twelve animals regardless of the sex divided equally two age periods (1, 15 days age). The adrenal glands were collected and fixed in 10% neutral buffered formalin for cortical tissue and Orth's solution for medullary tissue. The sections of 6 µm thicknesses were stained by Haemotoxyllin and Eosin stain (H&E), periodic acid Schiff stain (PAS) and Van Gieson stains. These specimens were conducted for histomorphologic investigation applied in both age periods. Anatomically the glands of local rabbits were paired white to creamy in color, oval to crescent in shape. It was embedded in fat, lying cranial to the cranial pole of kidney within the retroperitoneal cavity similar to these of other mammals. The left adrenal gland was far off from the left kidney as a compared with the right gland. Histologically the adrenal capsule observed as one layer in the two age periods. The zona glomerulosa appeared as an arch of cells at sub capsular region, while the zona fasiculata and reticular zone were undifferentiated at day one old and gradually developed with age. Medulla composed of central vein surrounding by chromaffin cells.
Key words: adrenal gland, rabbit, cortex, medulla, sinusoid.

دراسة نسجية شكليائية تطورية للغدة الكظرية في الارنب المحلي عند عمر يوم واحد وعمر خمسة عشر يوم

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الخلاصة

الغدد الكظرية هي غدد صماوية معقده تنظم عمليات وظيفيه في جسم الحيوان. هذه الدراسة اجريت على غدد كظريه اخذت من ارانب محلية خلال فترتين مختلفتين بعد الولادة .اثنى عشر ارنبا بغض النظر عن الجنس قسمت بالتساوي على فترتان (15,1 يوما من العمر). تم جمع الغدد الكظرية وثبتت باستعمال محلول الفورمالين المتعادل للنسيج القشري ومحلول الاورث لنسيج اللب. اجري الفحص النسجي الشكلي على العينات في كل الفترات العمرية. الجزء التشريحي في هذه الدراسة قصد الى توصيف مكان الغده. الغدد الكظرية في الارنب المحلي هي زوج من الغدد البيضاء اللون الى كريمي، بيضوية الشكل الى هلالية ، مطمورة في النسيج الدهني ، تقع اماميا للقطب الامامي للكلية ضمن تجويف خارج الخلب كما في باقي الثدييات. الغده اليسرى بعيدة عن الكلية اليسرى بالمقارنة مع الغدة اليمنى. شمل الجزء النسجي تثبيت الغده الكظرية وعمل الطمر الروتيني بشمع البرافين. صبغت المقاطع النسجية التي سمكها 6 مايكرون بصبغات الهيموتوكسلين والايوسين وصبغة ملون شيف حمض البيروديك وصبغة فان كيسن. لوحظت محفظة الغدة الكظرية على شكل طبقة واحدة في هاتين الفترتين. النطاق الكبيبي لوحظ على شكل اقواس من الخلايا تحت منطقة محفظة الغدة اما النطاق الحزيمي والشبكي كانا غير متمايزين عند عمر يوم واحد وتدريجيا تطورا مع العمر. لب الغدة الكظرية يتكون من وريد مركزي محاط بخلايا الكرومافين.
الكلمات المفتاحية: الغدة الكظرية ، الارنب ، القشرة ، اللب ، الجيبانيات.

Introduction

Rabbits are small mammals in the family *Leporidae* of the order *Lagomorpha*, found in several parts of the world. There are eight different genera in the family classified as rabbits, such as the European rabbit (*Oryctolagus cuniculus*); cottontail rabbits (*genus Sylvilagus*) and Amami rabbit (*Pentalagus furnessi*) and others. The male is called a buck and the female is a doe; a young rabbit is a kitten or kit (1). Adult rabbits can eat up to 30% of their bodyweight of food in a single night. Their breeding season extends from January to August, when a succession of litters, usually of three to seven young can be produced by the does at 30 day intervals. Doe reach sexual maturity from as early as four months old (2). The two adrenal glands are named by their anatomical position cranially to the two kidneys (3). The mammals adrenal gland which investigated after birth was composed of two distinct functional compartments, i.e., the cortex and the medulla. It is the vital endocrine gland that occupies the central role in the regulatory mechanisms of the body metabolism. The parenchyma of the adrenal gland of birds and some animals constituted mainly of three components namely the cortical tissue, chromaffin tissue and vascular sinusoids (4, 5, and 6). This study was aimed to detect the differences between these two ages.

Materials and methods

Twelve healthy rabbits of two different ages (1, and 15 days age) were used in this study. All animals were examined before their euthanasia. The adrenal glands were examined grossly with aid of dissecting microscope. The euthanasia was conducted intramuscular injection of a mixture of ketamine HCl 60 mg/kg and xylazine 6 mg/kg then the abdominal cavity was opened and both right and left adrenal glands were exposed. Before removing of the adrenal gland, 10% neutral buffered formalin was injected through abdominal aorta and left for 48 hours for fixation of the organs.

Anatomical study: It included measuring the weight and length of each animal in different age periods. The current work includes: Position, weight, size and dimensions of adrenal gland was recorded.

Histological study: Half of samples washed with distilled water and put in suitable container with Orth's solution as a fixative containing potassium dichromate, sodium sulfate, formalin and water for 24 hours then specimens were transferred to 2.5% aqueous solution of potassium dichromate for 48 hours after that they wash by putting them under running water overnight and adding of 5 ml of acetic acid to it will improve this fixative for the demonstration of chromaffin granules in the medullary tissues (7). Another half of specimens were fixed with neutral buffer formalin to the cortical tissue of the adrenal gland. The staining method is done for differentiate different slide components. Hematoxylin & Eosin stain, Periodic Acid Schiff (PAS) stain, Van Gieson stain was used. Measurement of thickness of capsule, cortex, medulla, sinusoid by oculometer equipment.

Results

Anatomical study:

The anatomical observations of the current study indicated that at one and fifteen days old rabbits, the right and left adrenal glands regardless of their gender were found located in the abdominal cavity cranial to the cranial pole of the kidney. Both glands were white to creamy color positioned retroperitoneal and embedded in a mass of fat (Fig. 1, 2)**.** The shape of the adrenal gland appeared crescent in the left adrenal, while the right gland was an oval in the shape (Fig. 3). The cross section of the adrenal gland at all ages showed an outer pallor part which was the cortex and an inner dark brown part which was the medulla (Fig. 4).

Histological study:

Capsule: The adrenal glands were enclosed in the capsule which showed one layer of cells and collagen fibers at one and fifteen days of age**.** The capsule of the adrenal gland at one day characterized by thick collagenous fibrous connective tissue. It surrounded the adrenal gland and projected thick trabeculae

into the gland tissue (composed of fibers and undifferentiated cells) and spread to all zones of cortex and even to the medulla (Fig. 5). At fifteen days-aged rabbit there was wavy shaped border present between the capsule and cortex (Fig. 5, 6). The capsule consists of collagenous bundles and few fibroblasts with elongated nucleus near to cortex.

Zona glomerulosa: The outer most zone of the cortex located underneath the capsule, at day one of age represents 33.3% of the total cortical thickness (Table 11). Histological examination of the adrenal in this ages characterized by aggregation of small poly-hedral cells as arches beneath the capsule penetrated by thick trabeculae (Fig. 7, 8). These cells were possessed large amount of clear cytoplasm with small rounded basoph-ilic nuclei.

Zona fasiculata: It was wide and extended to occupy a large area of the cortex compared with the other two zones of cortex. It extended between upper zona glomerulosa and lower zona reticularis. At one day of age, it was still undifferentiated. According to that, measuring of the thickness of this was impossible due to the undifferentiated nature of its cells and its integration with zona reticularis. At fifteen days of age. The cells of this zone appeared foamy in appearance due to the presence of vacuoles in their cytoplasm (Fig. 9).

Zona Reticularis: Innermost zone of the adrenal cortex undergoes the similar morphological changes that were takes placed in the zona fasiculata at one day undifferentiated zone. While at fifteen days age, the cells of zona reticularis were arranged as net-work and contained large central nucleus (Fig. 10).

Medulla: It occupied the central part of the adrenal gland and composed of the medullary cells named chromaffin cells. Its characteristic feature was the presence of the central vein. Aggregations of cells like clusters contain secretory granules in their cytoplasm for epinephrine and nor epinephrine hormones production. As well as scattered through the cortex. At one day of age the medulla contain central vein with large diameter. This may be due to highly mitotic division occurred in the whole adrenal gland and this may in need to higher blood supply and at the same time needed a well venous drainage through central veins (Fig. 11). At fifteen days of age, the decrease in diameter of the central vein may be due to the heavy aggregation of chromaffin cells around the central vein causing the narrowing of its lumen (Fig. 12). The chromaffin cells were arranged in clustered or cords separated by sinusoids, around venules and central vein. Large numbers of sinusoids spread in the cortex and medulla.

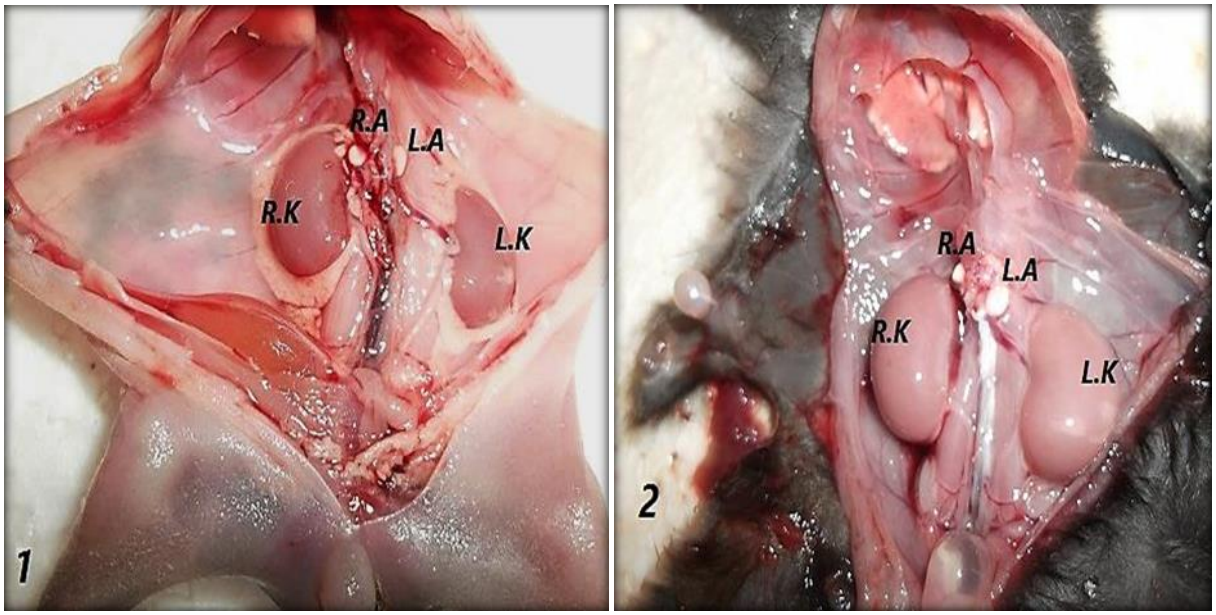
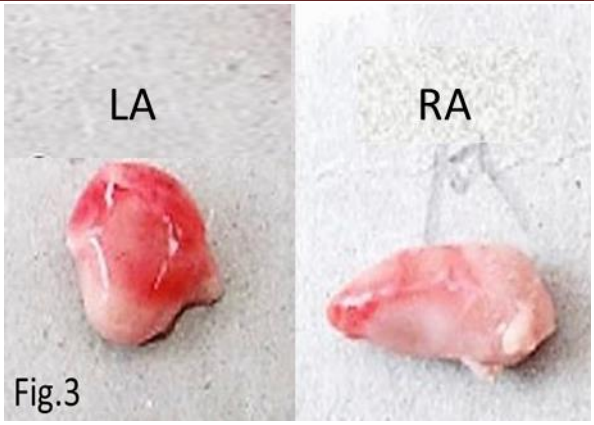


Fig. (1, 2): The adrenal glands in situ at 1 day (1), and 15 days old rabbits (2): Right adrenal gland (R.A), left adrenal gland (L.A), right kidney (R.K) and left kidney (L.K).



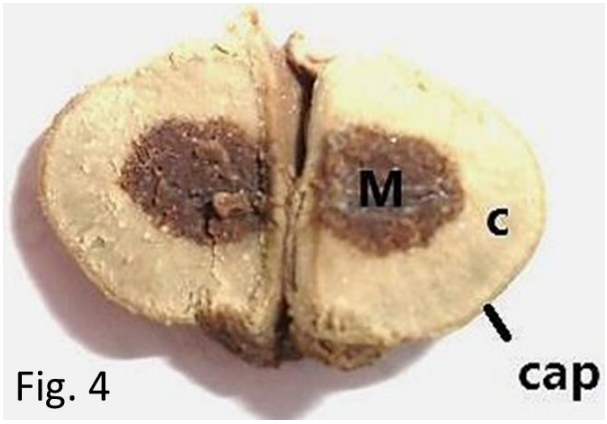


Fig. (3): The shape of adrenal gland in both ages. The left adrenal gland crescent in shape, and the right is an oval.

Fig. (4): Cross section of the adrenal gland. Capsule (cap), cortex(C), and medulla(M).

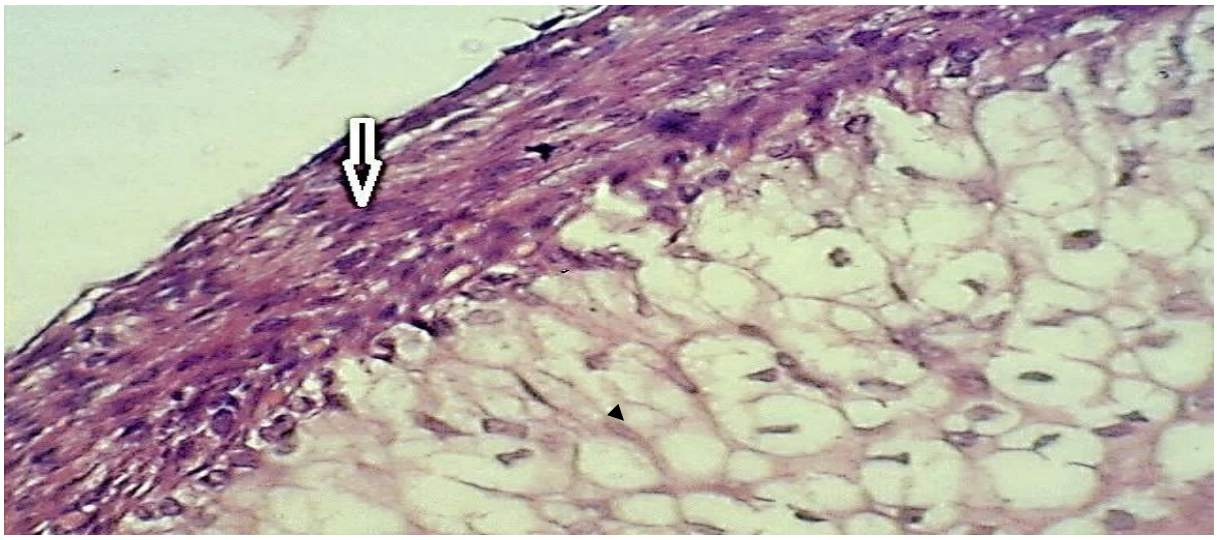


Fig. (5): Adrenal capsule of 1 day old-rabbit. Consist of one layer, bundles of collagen fibers and reticular cells (arrow) (H&E X1000).

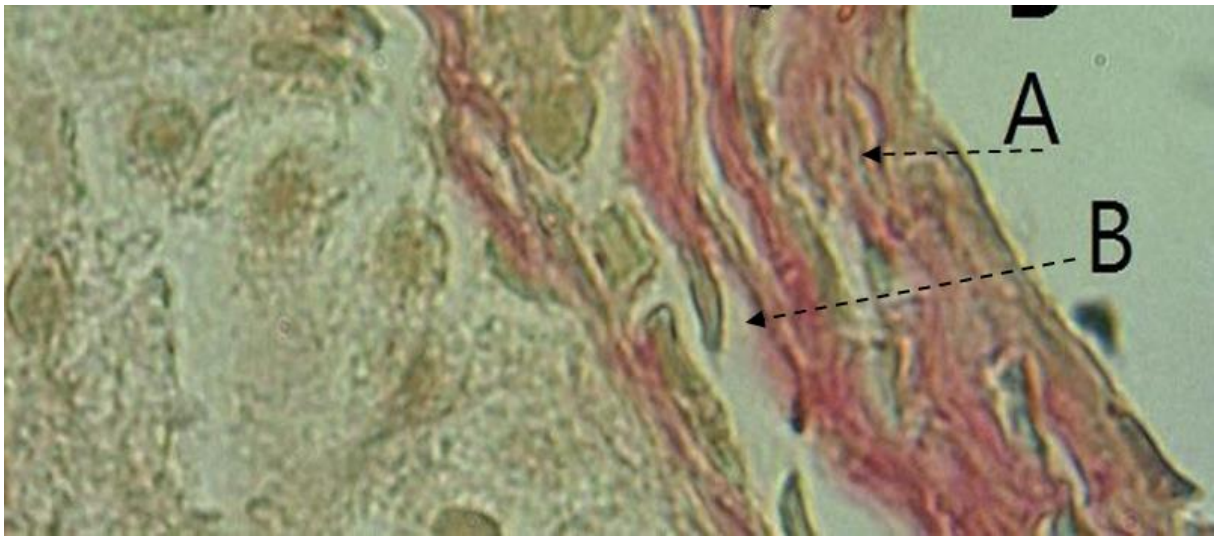


Fig. (6): Adrenal capsule of 15 days old rabbit. Show fibrous tissue (A) and Cellular elements (B) (Van Gieson stain X 1000).

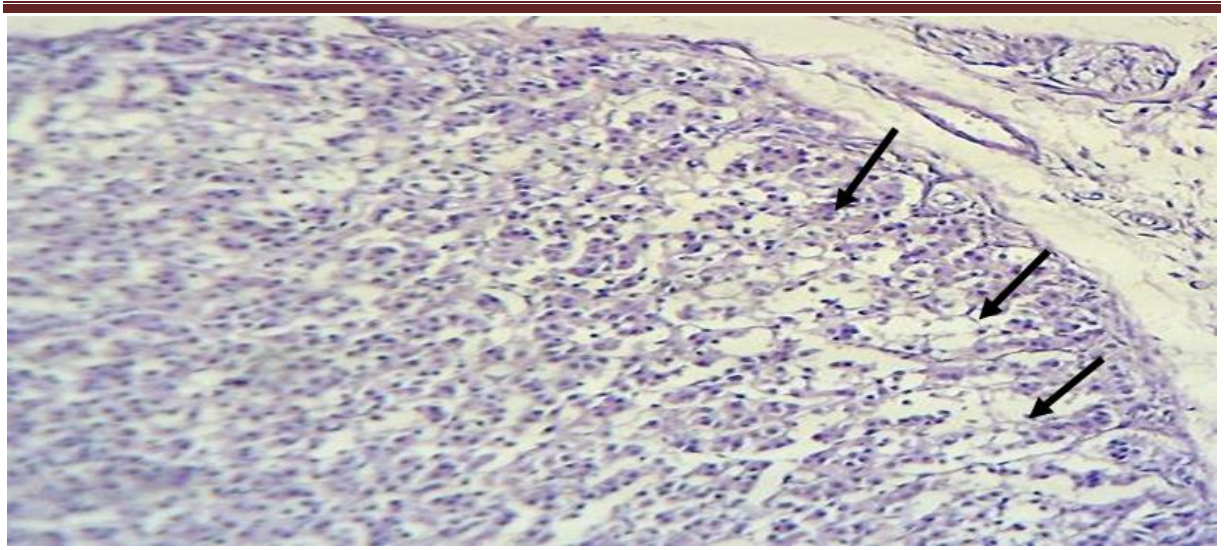


Fig. (7): Zona glomerulosa of 1 day old rabbit. Show zona glomerulosa cells (Black arrows) (PAS stain X100).

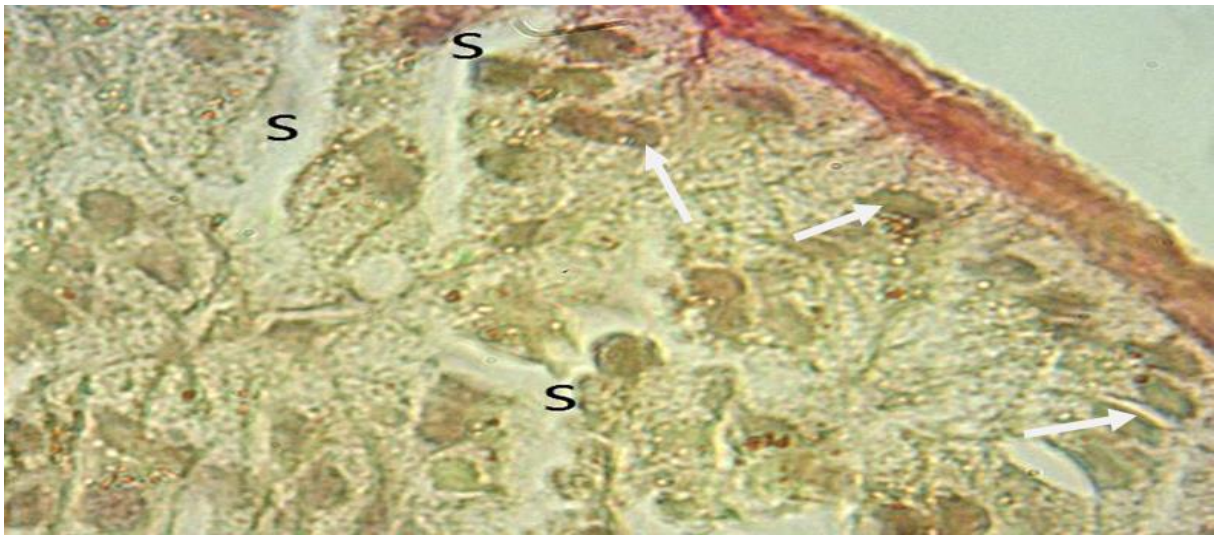


Fig. (8): Zona glomerulosa of 15 days old rabbit. Show zona glomerulosa cells (arrows), blood sinusoids (s) (Van Gieson stain X1000).

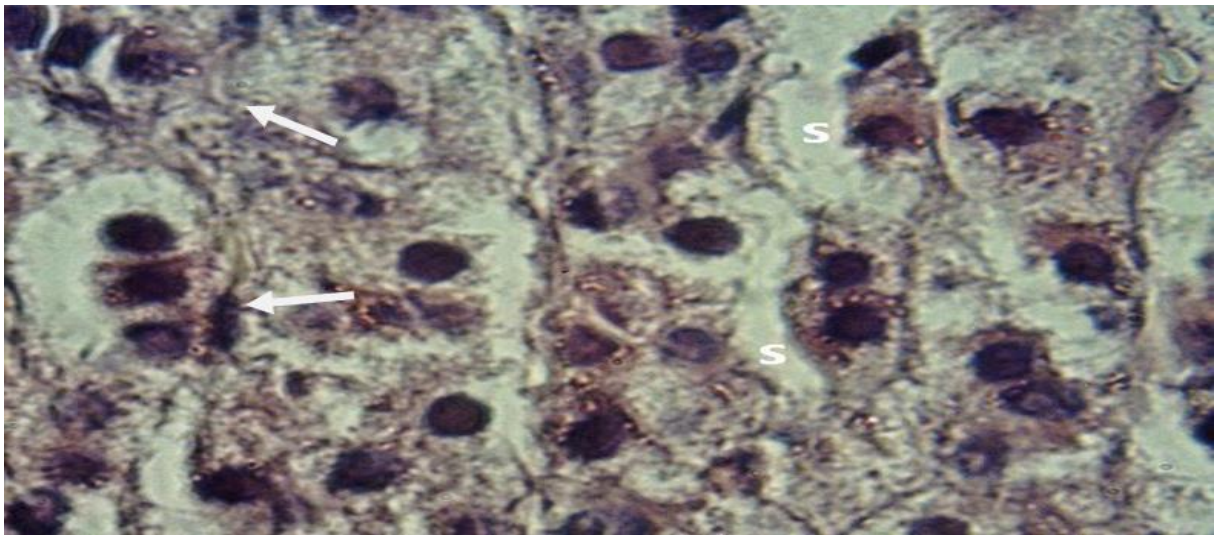


Fig. (9): Zona fasiculata of 15 days old rabbit. Show cords of basophilic cells (White arrows), blood sinusoids (s) (H&E X1000).

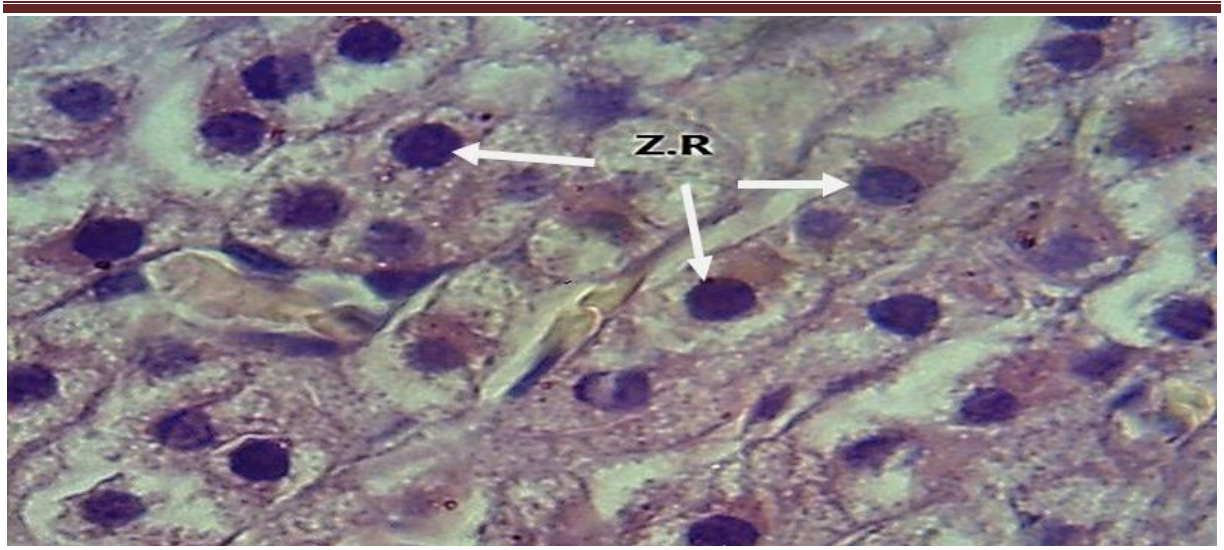


Fig. (10): Zona reticularis of 15 days old rabbits. Show the basophilic cells (white arrows) (H&E X1000).

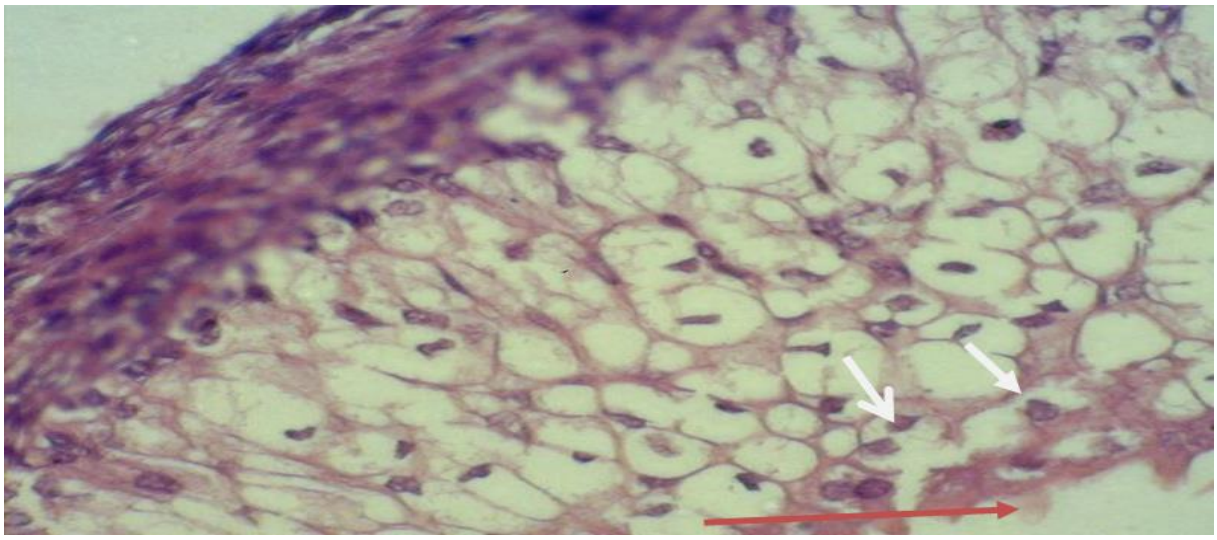


Fig. (11): Medulla of 1 day old rabbits. Showed the chromaffin cells (White arrows), central vein (red arrow) (H&E X400).

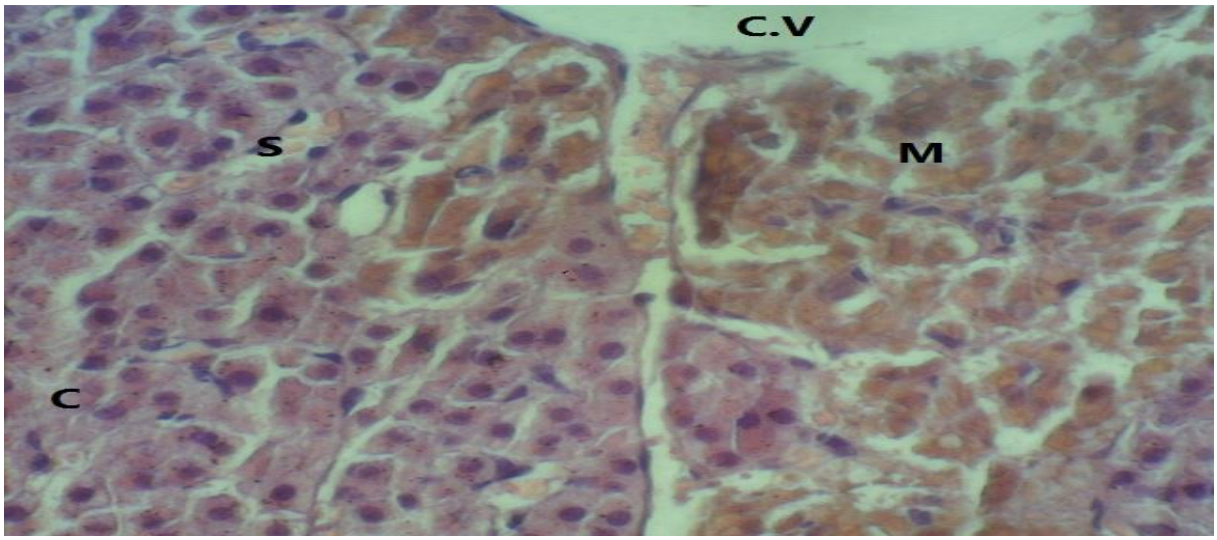


Fig. (12): Medulla of 15 days old rabbits. Show medulla (M), blood sinusoids (S), central vein (C.V), and cortex (C) (H&E X400).

Discussion

Anatomical study: Both glands were positioned retroperitoneal and embedded in a mass of fat located in the abdominal cavity cranial to the cranial pole of the kidney. The distance between the right adrenal gland and its related right kidney was lesser than the distance present between the left gland and the left kidney. It may be due to the pressure of adjacent organs. While the shape, color, weight character were previously observed in the bats (6) and rats too (8), (9), cats (10), (11).

Histological study: Capsule similar findings were previously found in the *Rattus norvegicus* (8) and rabbits (5). The thickness of the capsule at one day age increased due to the thought that one day age represented as intermediary stage between the pre-natal and post-natal periods. The increased in thickness was occurred on the expanse of the other cortical zones located underneath it. These results were in consistence with the previous findings in the rat's adrenal glands (12), (13), subsequently in goat (14) and rabbits (5). The capsule gradually became two layers outer (fibrous) and inner cellular. These observations were found in the adrenal of rat (15), goat (14) and rabbit (5). The Zona glomerulosa of the both ages was characterized by aggregation of small polyhedral cells as arches beneath the capsule. This histological result also observed and documented previously by (15) in the adrenal of albino rats, (16) in the adrenal of mice, (17) in the adrenal of male albino rats and (5) in the adrenal of the rabbit. The mitotic figures in this zone in all ages also observed by (18), (19), (20) and (13) in the adrenal glomerulosa of rat, these cells were possessed large amount of clear cytoplasm with small rounded basophilic nuclei these histological observations were found previously too by (21) in the adrenal glands of the Dolphin. In Zona fasiculata showed an important aspect through its development. It was wide and extended to occupy a large area of the cortex compared with the other two zones of cortex. It extended between upper zona glomerulosa and lower zona reticularis. Zona fasiculata in one day undifferentiated zone also occur in zona reticularis and then differentiated in 15 day age. The cells of this zone appeared foamy cells in appearance due to the presence of vacuoles in their cytoplasm. These vacuoles present as a result of dissolving of fat droplets with xylene through histological procedure of slide preparation. The cells contain oval or rounded dark stained basophilic nuclei and arranged as a cords of cuboidal or low columnar cells. These histological observations were previously mentioned by (17) in adrenal gland of the albino rats, (16) and (22) in mice, (20) in the porcupine and rat, respectively by (3) and (17) albino rat. The zona reticularis innermost zone of the adrenal cortex undergoes the similar morphological changes that was takes placed in the zona fasiculata at one day undifferentiated zone. The cells of zona reticularis were arranged as net-work and contained large central nucleus. This observation was mentioned too by (4) and (5) in the adrenal reticularis of the rabbit and (17) in the adrenal of the albino rat. This result agrees with those of (4), (13) in the rat; (12) in albino rat. Medulla It occupied the central part of the adrenal gland and composed of the medullary cells named chromaffin cells. Its characteristic feature mentioned similarly by (20) in the porcupine was the presence of the central vein, this histological feature was observed by many other investigators (23), (24), (25), (20) This result was mentioned by (15) in albino rat, (6) in Indian male fruit bat, (2) in Rhesus Macaque and (26) in human embryo and (20), (27). This result mentioned by (25), (3), (28), (29) and (30) in humans and some animals.

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