

## COMPARATIVE ANATOMICAL AND HISTOLOGICAL STUDY OF THE RABBITS (*Oryctolagus cuniculus*) ADRENAL GLAND AT THIRTY AND NINETY DAYS AGE.

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### ABSTRACT

Twelve animals regardless of the sex divided equally into two age periods (30, 90 days age). The adrenal glands were collected and fixed in 10% neutral buffered formalin for cortical tissue and Orth's solution for medullary tissue. These specimens were conducted for histomorphologic investigation applied in both age periods. The glands of rabbits were paired white to creamy in color, oval to crescentic in shape. It was embedded in fatty tissue, 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. Histological part included fixation of the adrenal glands and then processed by routine paraffin embedding. The sections of 6  $\mu\text{m}$  thicknesses were stained by H&E, PAS and Van Gieson stains. The adrenal capsule observed as one layer in the two age periods. The zona glomerulosa appeared as arches of cells at sub capsular region, while the zona fasciculata and reticular zone were developed with age. Medulla composed of central vein surrounding by chromaffin cells.

**Key words:** adrenal gland, rabbit, cortex, medulla, sinusoid.

### 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 ([Http://en.wikipedia.org/wiki/Rabbit](http://en.wikipedia.org/wiki/Rabbit)). 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 (McKillop *et al.*, 1993). The mammals' adrenal

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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 (Hassan, 1975; Fahmy *et al.*, 2008; Papadkar & Dhamani 2012).

## **MATERIALS AND METHODS**

Twelve healthy rabbits of two different ages were used in this study; those are 30, 90 day's age. The euthanasia was conducted intramuscular injection of ketamine HCl 60 mg/kg and xylazine 6 mg/kg, and after that the abdominal cavity was opened and both right and left adrenal glands were exposed. 10% buffer formalin was injected through abdominal aorta and left for 48 hours for through fixation of the organ. Anatomical study 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. Histologically: other 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 ( Luna,1968). 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.

## **RESULTS AND DISCUSSION**

Anatomically at thirty and ninety days old rabbits, the right and left adrenal glands regardless of their gender of the local rabbit were 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 adrenal gland appeared crescent in the left adrenal while the right gland is an oval in the shape (Fig. 3). The cross section of the adrenal gland at all ages showed an outer pallor part which is the cortex and an inner dark brown part which is the medulla (Fig. 4). Histologically: the adrenal glands were enclosed in the capsule which showed two layers (outer fibrous and inner cellular) at thirty and ninety days of age (Fig. 5).

**Zona glomerulosa**

At thirty days of age, the zona glomerulosa was well differentiated and contains many sinusoids. The cells were polyhedral in shape with relatively large oval to rounded nuclei with relatively small amount of cytoplasm, the cells arranged as arches under capsule, the trabeculae appear thin and it showed many mitotic figures (Fig.6). At ninety day age the zona glomerulosa the cells have large rounded nuclei and less amount of cytoplasm. The sinusoids were well observed beside the cellular proliferation of mitotic division among the cells (Fig.7).

**Zona fasciculata**

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. The cells of this zone appeared foamy in appearance due to the presence of vacuoles in their cytoplasm. The zone composed of cellular cords, the cells showed foamy picture due to the presence of vacuoles in their cytoplasm. The examination showed high proliferation with containing small rounded basophilic nuclei, the cells contain oval or rounded darkly stained basophilic nuclei and arranged as a cords of cuboidal or low columnar cells (Fig. 8). Zona fasciculata at ninety days old rabbits was well developed Histological picture was similar to that of the thirty day of age (Fig. 9).

**Zona Reticularis**

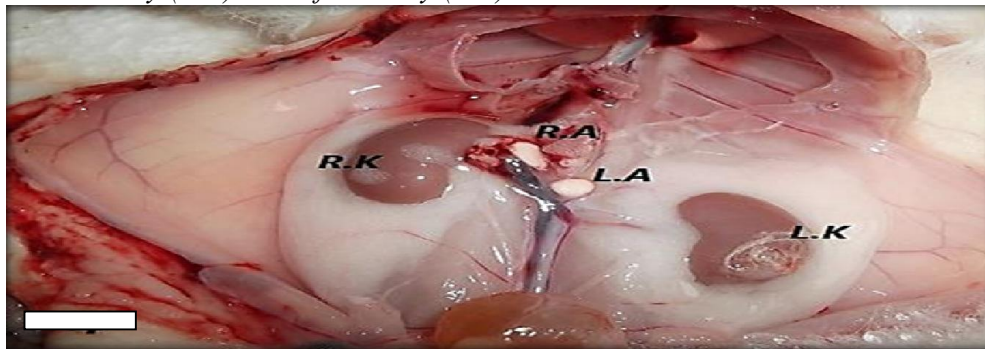
It composed of large polyhedral cells with oval or rounded basophilic nuclei and showed different degrees of mitotic division and the presence of many sinusoids in 30 days. Clear obvious boundaries and clear demarcation among its cells (Fig 10). There were no clear demarcations between zona reticularis and medulla but the cells of both reticular zone and medulla overlap with each other due to development by migration of chromaffin cells to the center of the adrenal gland (Fig. 11).

**Medulla**

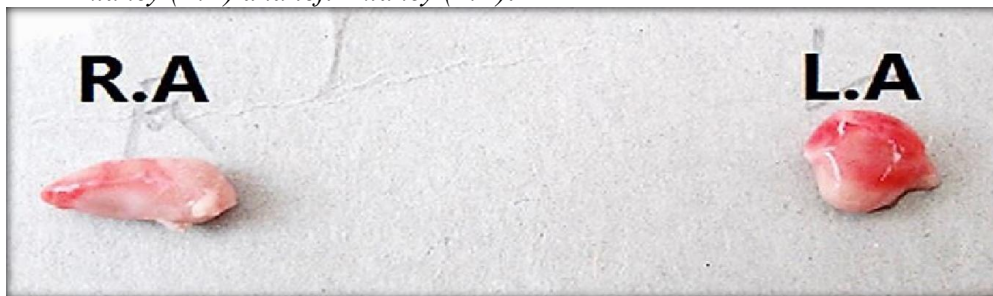
It occupied the central part of the adrenal gland and composed of the medullary cells named chromaffin cells. Its characteristic feature was the presence of the central vein. Chromaffin cells arranged in cellular cords or clusters surrounding the central vein. These cells possessed either epinephrine or nor epinephrine secretory granules. The chromaffin cells stained brown when fixed with potassium dichromate salts (Fig.12).while the histological observation in ninety days age and the constant development of adrenal gland and the chromaffin cells have a large central nuclei and granular cytoplasm (Fig.13).



**Fig. 1:** Adrenal glands in situ of 30 days old-rabbits, respectively. It showed right adrenal gland (R.A), left Adrenal gland (L.A), right kidney (R.K) and left kidney (L.K)



**Fig. 2:** Adrenal glands in situ of 90 days old-rabbits, respectively. It showed right adrenal gland (R.A), left Adrenal gland (L.A), right kidney (R.K) and left kidney (L.K).

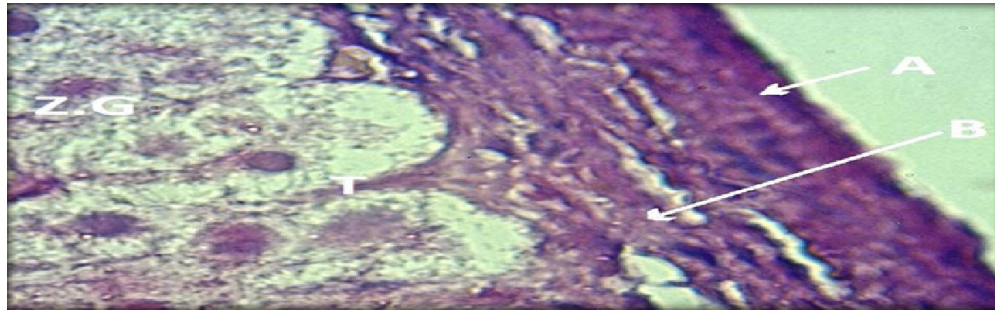


**Fig.3:**The shape of adrenal gland. It showed a crescent shape in the left adrenal while the right gland is an oval in shape.

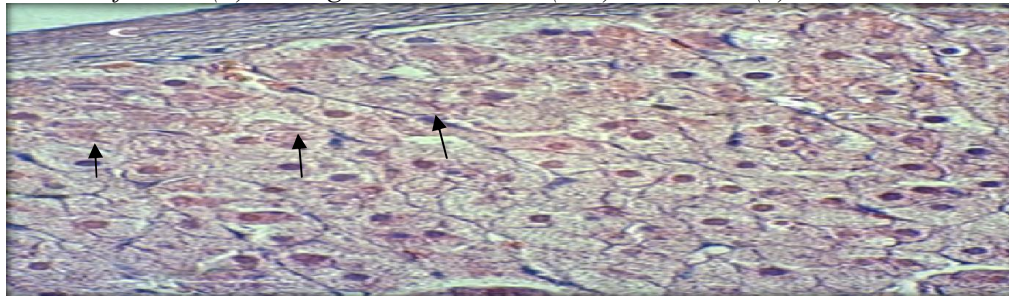


**Fig.4:**Cross section of the adrenal gland in rabbit showed capsule (cap), Cortex(C) and medulla (M).

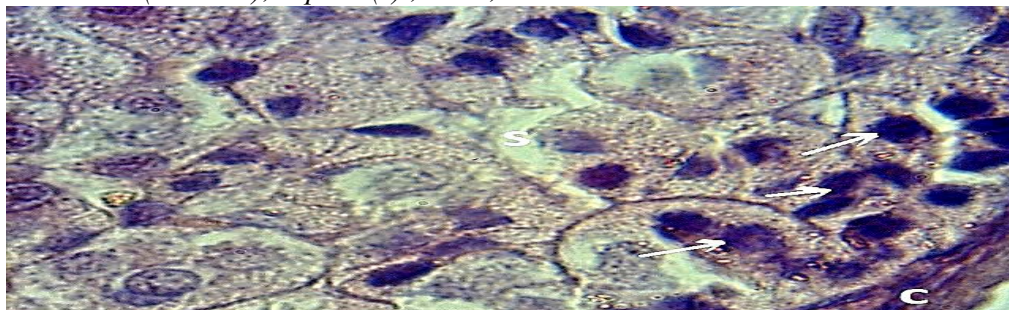




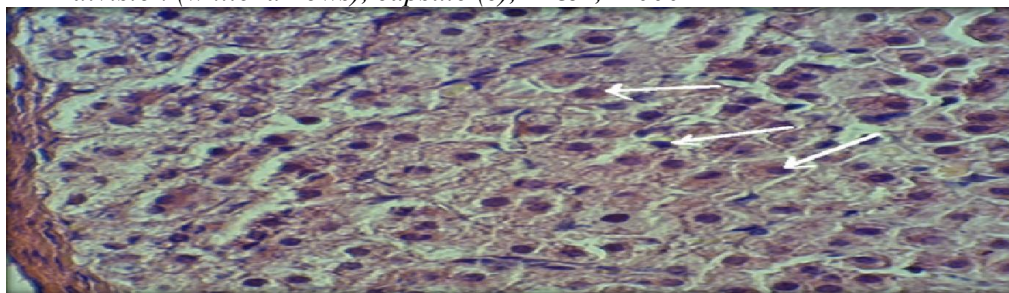
**Fig.5:** Capsule of 30 days -old rabbits. It showed two Capsule cellular (B) and fibrous (A), zona glomerulosa cells (Z.G), trabecula (T), PAS,X 1000



**Fig.6:** Zona glomerulosa of 30 days -old rabbits. It showed zona glomerulosa cells ( arrows), capsule(c) , H&E,X400

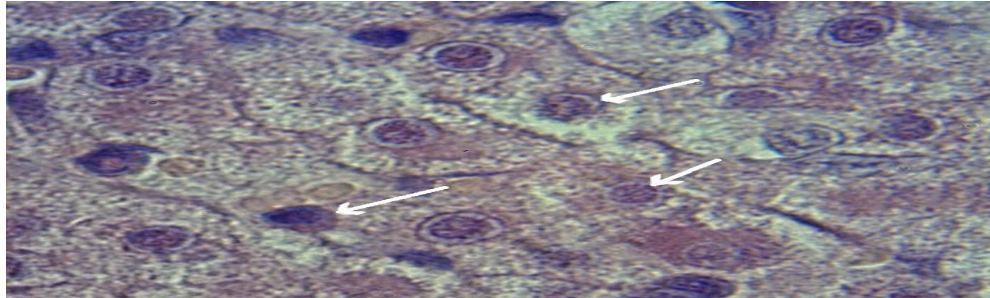


**Fig.7:** Zona glomerulosa of 90 days -old rabbits. It showed Mitotic division (white arrows), capsule (c), H&E,X1000



**Fig.8:** Zona fasciculata of 30 days -old rabbits. It showed cords of basophilic cells (white arrows),capsule(red arrow), H&E, X400

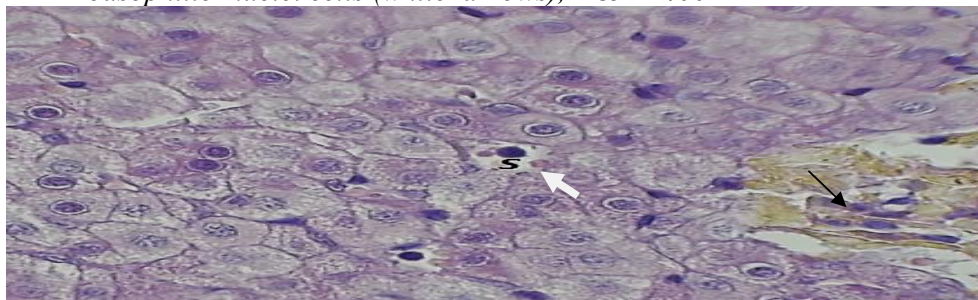




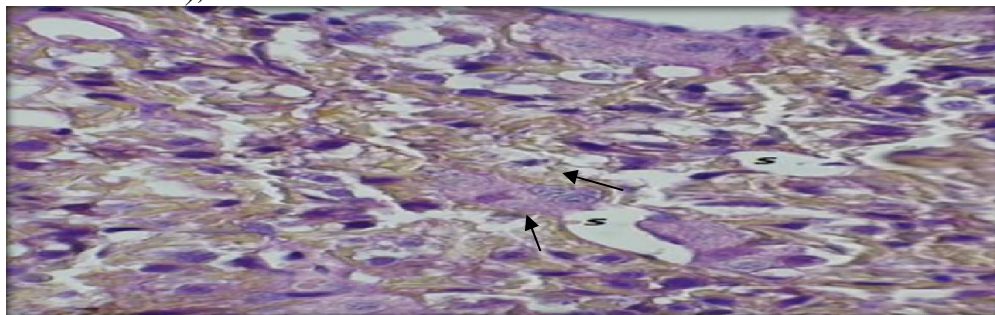
**Fig.9:***Zona fasciculata of 90 days -old rabbits. It showed cords of basophilic cells (white arrows), H&E, X1000*



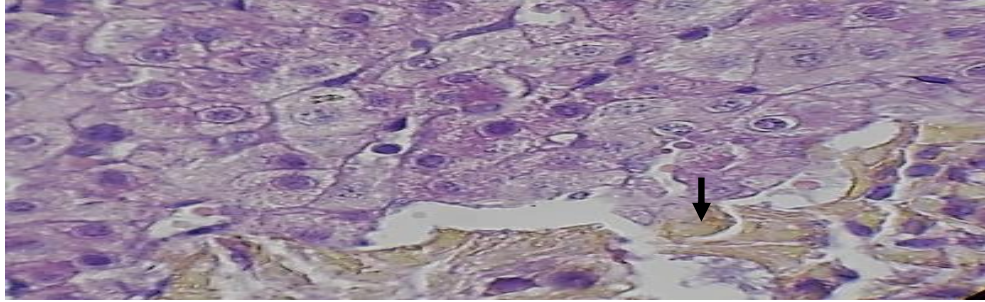
**Fig.10:***Zona reticularis of 30 days -old rabbits. It showed the network of basophilic nuclei cells (white arrows), H&E X400*



**Fig.11:***Zona reticularis of 90 days -old rabbits. It showed the network of basophilic nuclei cells (white arrow), and medulla(black arrows),PASX1000*



**Fig.12:***Medulla of 30 days -old rabbits. It showed the chromaffin cells (arrows), H&E, X1000*



**Fig.13:** Medulla of 90 days -old rabbits. It showed the chromaffin cells (Arrow), PASX400, H&E, X1000

### 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 maybe to shortage of renal or adrenal artery. While the shape, color, weight character were previously observed in the bats (Papadkar& Dhamani, 2012) and rats too (Ezumi *et al.*, 2007). Gilbert, 2004), in cats (Westropp *et al.*, 2003; Tomich, 1965).

### Histological study

Capsule similar findings were previously found in the *Rattus norvegicus* (Ezumi *et al.*, 2007)) and rabbits (Fahmy *et al.*, 2008).The thickness of the capsule at 30 days age gradually became two layers outer (fibrous) and inner cellular. These observations were found in the adrenal of rat (El Sayed *et al.*, 1990); goat (Dimova, 2003) and rabbit (Fahmy *et al.*, 2008). 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 (El Sayed *et al.*, 1990) in the adrenal of albino rats, (Rosol *et al.*, 2001) in the adrenal of mice, (Hafez *et al.*, 2005), in the adrenal of male albino rats and (Fahmy *et al.*, 2008) in the adrenal of the rabbit. The mitotic figures in this zone in all ages also observed by Lombarado & Cortesini (1990); Chen *et al.* (1998); Yilmaz & Girgin (2005) and Mughal *et al.* (2004) 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 Clark *et al.* (2005) in the adrenal glands of the Dolphin. In Zona fasciculata 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 fasciculata cells 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 darkly stained basophilic nuclei and arranged as a cords of cuboidal or low columnar cells. These histological observations were previously mentioned by Hafez *et al.* (2005) in adrenal gland of the albino rats Rosol *et al.* (2001) ; Bielohuby *et al.*, (2007) in mice, (Yılmaz and Girgin, 2005) in the porcupine and rat, respectively by Aughey & Frye (2011) and (Hafez *et al.* (2005) albino rat. Zona Reticularis innermost zone of the adrenal cortex undergoes the similar morphological changes that were taking place in the zona fasciculata at one day undifferentiated zone. The cells of zona reticularis were arranged as network and contained large central nucleus. This observation was mentioned too by Junqueira and Carneiro (1980) and Fahmy *et al.* (2008) in the adrenal reticularis of the rabbit and Hafez *et al.* (2005) in the adrenal of the albino rat. This result agrees with those of Junqueira and Carneiro (1980), Mughal *et al.* (2004) in the rat; (El shennawy and Aboelwafa (2011) in albino rat. Medulla occupied the central part of the adrenal gland and composed of the medullary cells named chromaffin cells. Its characteristic feature mentioned similarly by Yılmaz and Girgin(2005) in the porcupine was the presence of the central vein, this histological feature was observed by many other investigators Coupland & MacDougall, 1966; Lemos *et al.*, 2006; Mescher, 2010; Yılmaz and Girgin, 2005. This result was mentioned by El Sayed *et al.* (1990) in albino rat, Papadkar & Dhamani (2012) in Indian male fruit bat Lemos *et al.* (2006) in Rhesus Macaque and Papadkar & Dhamani ( 2012) in human embryo and Yılmaz & Girgin, (2005); (Chavhan *et al.*(2011). This result mentioned by Mescher, (2010); Aughey & Frye (2011); Junqueira and Carneiro (1980), Wheater *et al.* (1987) and Junqueira *et al.* (1998) in humans and some animals.

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## دراسة نسجية تشريحية مقارنة للغدة الكظرية في الارنب عند الثلاثين والتسعين يوماً من العمر.

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## المستخلص

قسم اثنا عشر أرنباً بغض النظر عن الجنس بالتساوي إلى فترتين عمريتين هي 90 ، 30 يوماً. جمعت الغدة الكظرية وثبتت في 10% محلول الفورمالين المتعادل للنسيج القشري ومحلول الاورث لنسيج اللب. هذه العينات نقلت للاستقصاء النسجي الشكلي الذي طبق في كلتا الفترتين العمريتين. الغدة الكظرية في الارنب هي زوج من الغدد البيضاء الى كريمي اللون ، بيضوية إلى هلالية الشكل، مطمورة في النسيج الدهني ، تقع أمامياً للقطب الأمامي للكلية ضمن تجويف خارج الخلب كما في باقي الثدييات. الغدة اليسرى بعيدة عن الكلية اليسرى بالمقارنة مع الغدة اليمنى. الجزء النسجي اشتمل على تثبيت الغدة الكظرية بعد ذلك تمت معاملتها بالطمر الروتيني بشمع اليرافين. المقاطع النسجية التي سمكها 6 مايكرون صبغت بصبغات الهيموتوكسلين والايوسين وصبغة ملون شيف حمض البيروديك وصبغة فان كيسن. محفظة الغدة الكظرية شوهدت كطبقة واحدة في الفترتين العمريتين. النطاق الكببي ظهر كاقواس من الخلايا تحت منطقة محفظة الغدة بينما النطاق الحزيمي والنطاق الشبكي تطورا مع العمر. لب الغدة الكظرية يتألف من وريد مركزي محاط بخلايا الكرومافين.

الكلمات المفتاحية : الغدة الكظرية ، الأرنب ، قشرة الكظرية ، لب الكظرية ، الجيانيات.