

Histomorphological and Histochemical Study of Adrenal Gland in Adult Male of Guinea Pigs (*Cavia porcellus*)

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Received: 26/02/2019

Accepted: 06/05/2019

Publishing: 04/08/2019

Summary

The present study was carried on twenty adult males of Guinea pigs to evaluate the histomorphological and histochemical features of adrenal gland. Morphologically, Guinea pigs had a couple adrenal glands brown in color were located craniomedially attached to each cranial pole of kidney, the right adrenal gland had pyramidal shape, while the left had elongated shape. The statistical results showed that there is significant difference between the right and left adrenal gland in the weight, length, thickness, width and volume. The histological and histochemical results showed that adrenal gland was composed of cortex and medulla. The cortex was consisted of three zones. The zona glomerulosa was consisted of columnar or cuboidal cells arranged in groups as glomeruli, the zona fasciculata and reticularis were composed mainly of polygonal cells arranged in regular and irregular cords. The medulla region was composed of ovoid group of cells (chromaffine cells) that arranged in irregular cords separated by blood sinusoid and surrounded by central vein, contained two types of cells, the first cell was columnar in shape and brownish in color representing the epinephrine secreting cell, the second cell was polygonal in shape and light brownish in color with spherical nucleus representing the Norepinephrine secreting cell when fixed in chromate salts. All parts of adrenal gland exhibited strong to moderate reaction for Periodic Acid Schiff stain. The statistical results showed that there is significant difference between the right and left adrenal gland in the thickness of capsule, zona glomerulosa, zona fasciculata, zona reticularis and medulla region.

Keywords: Histological, Histochemical, Adrenal gland, Guinea pigs.

Introduction

Rodents are one of the largest orders of mammals, most rodents are herbivorous but some are omnivorous and some prey on insects. Guinea pig (*Cavia porcellus*) is small laboratory animals, which constitutes a small suborder (Hystricomorphic) belong to the family Caviidae and the genus *Cavia* were probably first introduced into Europe from South America (1). The Guinea pigs are excellent experimental subjects for physiological and histological studies due to many reasons, their body weight, size, ease to be handled and they adapt rapidly in laboratory situation. The main diet of Guinea pigs is the grass (2).

The adrenal glands are paired compound endocrine organ located in the crania-medial side of each kidney (3). They are playing important role for secrete many kinds of hormones, chemical substances which help to

processes as metabolism, water and electrolyte balance, sexual development, function of the immune system, and the stress response including cortisol, adrenaline, androgens and aldosterone(4).

The adrenal gland shows distinct species variations in shape, weight and size amongst domestic animals. It also varies in morphological maturation with age amongst mammals (5). It is generally made of two layers: The outer layer, with and radiated columns (cortex) and the internal cortex, presenting a medulla (marrow). They have different origins during embryogenesis (6).

The adrenal cortex is subdivided into three or four zones of epithelial cells, the zona glomerulosa is the outermost zone, the zona fasciculata is the widest zone of cortex and the innermost zone of adrenal cortex is zona reticularis (7). The data which may be obtained from the histomorphological and histochemical

investigations will provides basic scientific information to conduct physiological and pharmaceutical researches that are related to the diseases of adrenal glands, certainly the obtained data will provide good animal model for both veterinary field in animals and public health in human. Therefore the present investigation was aimed to: Throw a spot of light on the histological and histochemical structure of the adrenal gland in Guinea pigs (*Cavia porcellus*).

Materials and Methods

Twenty healthy adult males of Guinea pigs (*Cavia porcellus*) were used, the mean weight of the guinea pigs was 712.90 ± 13.01 gm. The animals were brought from a local commercial market of animals in Baghdad city, kept alive in plastic cages in animals house in department of anatomy and histology/ College of Veterinary Medicine/ University of Baghdad, given feed and water ad libitum and acclimated for two weeks before the research. The animals euthanized by using inhalation of chloroform in a closed container (8). Guinea pigs were humanly sacrificed and adrenal gland was dissected out. The shape, color and location were recorded before the fixation and the photographs of the adrenal gland was taken to depict the gross anatomy by using digital camera Sony cyber-shot (14.2 mega pixel).

Histological and histochemical study samples of adrenal gland was fixed in 10% neutral buffered formaldehyde and Orth's stock solution contain potassium dichromate to determine medulla of adrenal gland for 48hours then the tissues dehydrated by passing them through a series of up grading alcohol of the following concentrations (70%, 80%, 90% and 100%) then cleared in xylene and embedded in two changes of paraffin. The paraffin blokes were sectioned serially at $5\mu\text{m}$. Sections were stained with Hematoxylin and eosin and Masson Trichrome stain, for histochemical studies used Periodic Acid Schiff stain to detected the general carbohydrates(9). The histological sections of this study were examined by using light microscope type (Olympus/ Japan), with different magnifications (X4; X10 and X40), then the sections were photographed by using (Olympus/ Japan) microscope and digital

camera. An ocular micrometer calibrated with stage micrometer was used for histological parameters which include the thickness of capsule, cortex, zona glomerulosa, zona fasciculata, zona reticularis and medulla [10]. By using the Statistical Analysis System program, the data were expressed as mean \pm standard errors (SE) and p- value < 0.05 was considered statistically significance(11).

Results and Discussion

The anatomical results of this study showed that the Guinea pigs had a couple of adrenal glands of brownish color, that were located at craniomedially attached to each cranial pole of kidney (Fig. 1) as described in the rodents as in Viscacha (*Lagostomus maximus*)(12) and plum mouse (13) and these results discord with [14] who found that the adrenal gland of the Mexican Pack rat was light grey in color and located at the middle of the upper end of the kidneys .

In Guinea pigs, the right adrenal gland had pyramidal shape and the left adrenal gland had elongated shape (Fig. 2) as described in Spix's yellow-toothed (*Galea spixii*, Wagler, 1831) (15), but these finding disagree with (16)who found that the adrenal of guinea pig was bean-shaped in adult female and oval to egg-shaped in adult female hamster, the disagreement may be related to the body plane.

The statistical findings revealed that the means of weight, length, thickness and volume of left adrenal gland were higher (significant) than those of right adrenal gland at $P < 0.05$, these results were similar to (17) in south Indian gerbil. While the mean width of the right adrenal gland was higher (Significant) than width of the left adrenal gland this coincided with (12) in Viscacha (*Lagostomus maximus maximus*) (Table,1).

The histological and histochemical results showed that adrenal gland in Guinea pigs had the same histological structure of mammalian which composed mainly of inner region was the medullar and the outer region was the cortex associated with capsule (Fig. 3) as described by (18) in Rodents. In Guinea pigs, both right and left adrenal glands were surrounded by thick dense irregular connective tissue capsule; had a network of blood vessels, there were no muscle fibers in capsule of

adrenal gland. The connective tissue of capsule penetrated into the parenchyma and formed scattered collagen fibers in parenchyma was very clear in zona glomerulosa (Fig. 4) these results coincide with (19) in Rats .

The capsule of adrenal gland was exhibited positive reaction for Periodic Acid Schiff(PAS) stains imply that the capsule is rich in collagen and carbohydrate (Fig.5) as described by(20).

The cortex was divided into three zones: the outer was glomerulosa, which was associated with the capsule of organ; the fasciculata in the middle and reticularis was the inner zone of cortex, which was associated with medullar region (Fig. 3) as described in rodents by (12). In Guinea pigs there is a clear line of demarcation between zona glomerulosa and zona fasciculata, it was composed of columnar and cuboidal cells with dark spherical nuclei and acidophilic cytoplasm with little amounts of lipid vacuoles. These cells were arranged in regular band of glomeruli separated from each other by fine connective tissue (Fig. 6) these results conform to (21) in Guinea pigs.

The zona fasciculata of adrenal glands was composed of two parts, the outer part was consisted of polygonal cells arranged in parallel cord of one cell thickness, some cells were contained dark spherical nucleus and some contained light spherical nucleus, the cytoplasm was acidophilic and contained fewer amount of lipid vacuoles while in the inner part, the cells were arranged in irregular cords separated with blood sinusoid and this part contained large cells called spongiocytes with numerous lipid vacuoles (Fig. 7 and 8), the same results were reported by (22) in Guinea pigs and (23) in adult male greater cane rats, these results disagree with[16] who

revealed that the zona fasciculata in hamster had cuboidal cells with small vacuoles in the outer cells of this zone.

Zona glomerulosa and zona fasciculate was occurred moderate reaction for PAS stain as described by (24) in African giant rat due to present of carbohydrate in basement membrane of glomerular cells (Fig.5). The zona reticularis in both adrenal glands of Guinea pigs was composed of small polygonal cells arranged in irregular cords and separated by wide sinusoid and connective tissues (trabeculae), some of polygonal cells were contained small dark stain nuclei and other cells were contained large light stain nuclei (Fig.9 and 10).these results agree with (25) in domestic rabbits.

The medulla in Guinea pigs was composed of ovoid group of cells (chromaffine cells) that arranged in irregular cords separated by blood sinusoid and surrounded by central vein, contained two types of cells, the first cell was columnar in shape and brownish in color with spherical nucleus located at base of cell this representing the epinephrine secreting cell, the second cell was polygonal in shape and light brownish in color with spherical nucleus located in the center of cell this representing the Norepinephrine secreting cell (Fig.11-13), these results were similar to those reported by (24) in African giant rats and (22) in Guinea pigs.

Adrenal medulla was appeared positive reaction for the(PAS)(Fig. 14) this agree with (20) in African giant rats .

The statistical findings revealed that there is significant difference in the mean thickness of capsule, cortex, cortical zones and medulla between the right and left adrenal gland (Table, 2) due to different in function of the gland.

Table, 1: Anatomical parameters of right and left Adrenal gland in Guinea pigs.

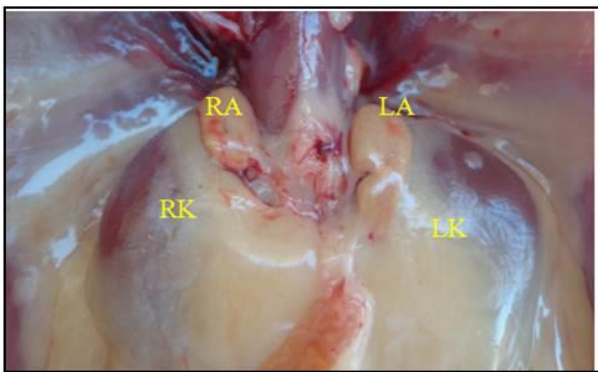
Type of animal	Anatomical parameters	Right adrenal gland	Left adrenal gland	T-Test
		Mean ± SE	Mean ± SE	
Guinea pigs	Weight (g)	0.428 ± 0.009	0.544 ± 0.008	0.0277 *
	Length (mm)	11.29 ± 0.06	13.65 ± 0.07	0.2063 *
	Width (mm)	7.20 ± 0.01	6.10 ± 0.02	0.0453 *
	Thickness (mm)	5.03 ± 0.11	5.14 ± 0.02	0.2349 NS
	Volume (ml)	0.340 ± 0.016	0.460 ± 0.016	0.0485 *

* (P<0.05) significant, NS: Non-Significant.

Table, 2: Histological parameters of right and left Adrenal gland in Guinea pigs.

Type of animal	Histological parameters(μm)	Right adrenal gland	Left adrenal gland	T-Test
		Mean \pm SE	Mean \pm SE	
Guinea pigs	Thickness of capsule	32.93 \pm 0.31	37.71 \pm 0.21	0.833 *
	Thickness of cortex	2149.64 \pm 2.25	1813.43 \pm 0.79	5.21 *
	Thickness of zona glomerulosa	51.78 \pm 0.40	64.85 \pm 0.30	1.104 *
	Thickness of fasciculate	1497.14 \pm 1.48	1249.29 \pm 1.30	4.31 *
	Thickness of reticularis	600.71 \pm 1.70	500.00 \pm 1.54	5.002 *
	Thickness of medulla	498.57 \pm 2.25	645.14 \pm 1.05	5.47 *

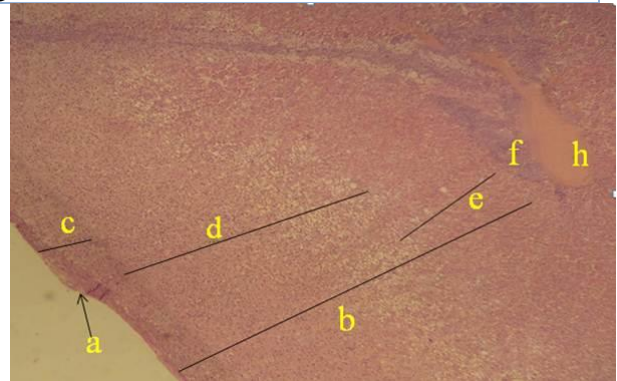
* (P<0.05) significant.



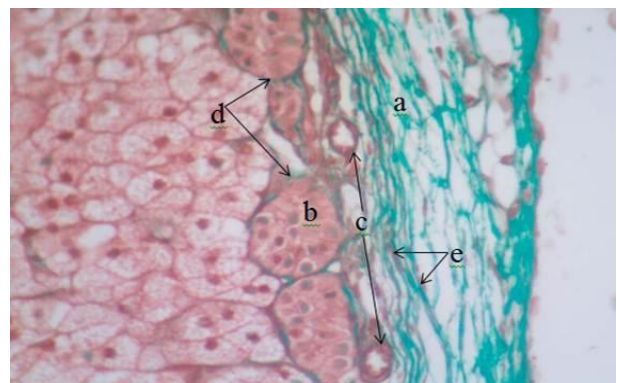
Figure, 1: Shows the gross anatomy of adrenal gland in Guinea pigs: Right adrenal- RA, LA- left adrenal, Right kidney- RK, left kidney- LK.



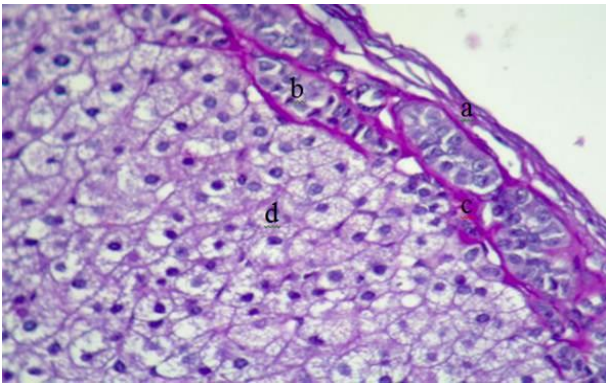
Figure, 2: Shows the gross anatomy of adrenal gland in Guinea pigs: Right adrenal gland- R, left adrenal gland-L.



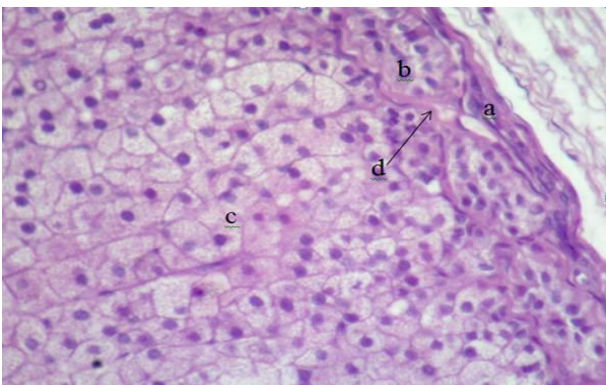
Figure,3: Adrenal gland in Guinea pigs shows: Capsule (a), Cortex (b), Zona glomerulosa (c), Zona fasciculata (d), Zona reticularis (e), Medulla (f), Central vein (h), H&E stain, X40.



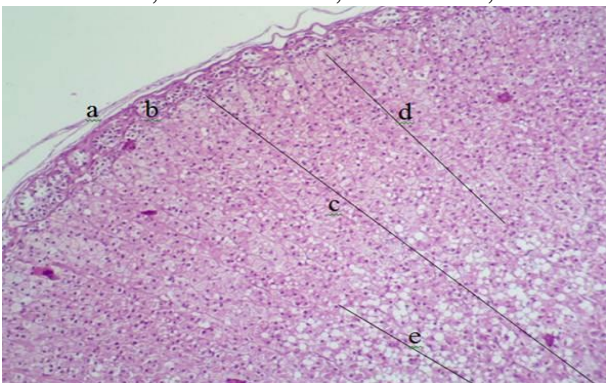
Figure, 4: Adrenal gland in guinea pigs shows: Capsule-a, Zona glomerulosa-b, Blood vessels-c, Trabeculae-d, Fibroblast-e, Masson Trichrome stain, X400..



Figure,5: Adrenal gland in Guinea pigs shows: Capsule-a, Zona glomerulosa-b, Trabeculae-c, Zona fasciculata-d, PAS stain, X400.

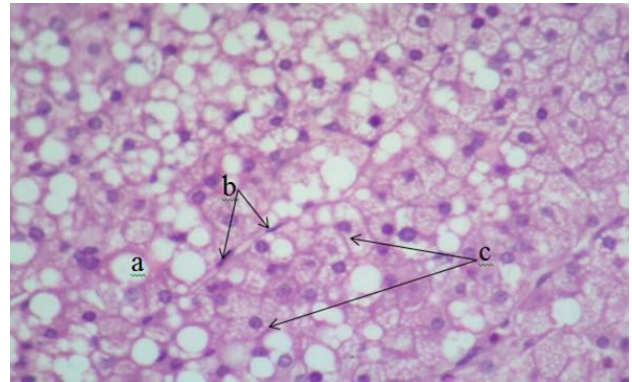


Figure,6: Adrenal gland in guinea pigs shows: Capsule-a, Zona glomerulosa-b, Zona fasciculata-d, Trabeculae-c, H&E stain, X400.

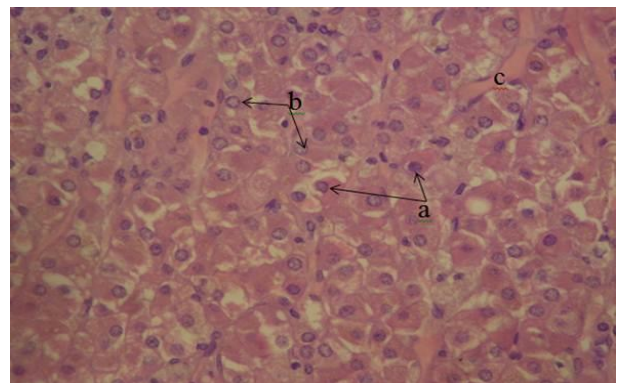


Figure,7:Adrenal gland in Guinea pigs shows: Capsule-a, Zona glomerulosa-b, Zona fasciculata-c, Outer part of zona fasciculata-d, Inner part of zona fasciculata-e,H&E stain,

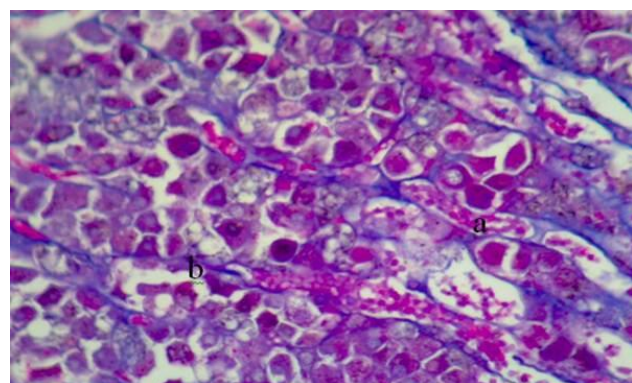
X100.



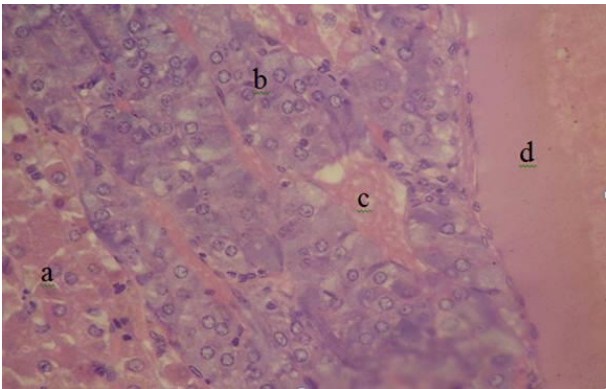
Figure,8: Inner part of zona fasciculata of adrenal gland in guinea pigs shows: Spongiocytes-a, Endothelial cell of blood sinusoids-b, Polygonal cells-c, H&E stain, X400.



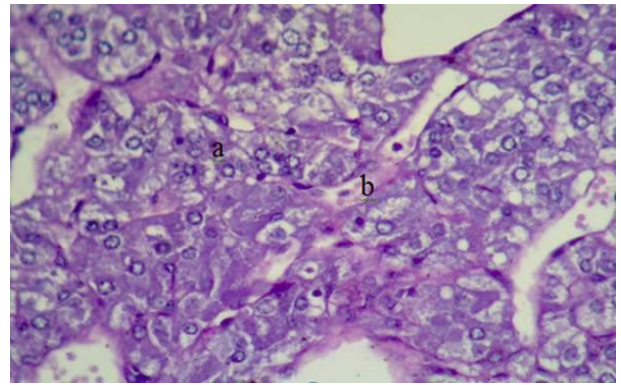
Figure,9: Zona reticularis of adrenal gland in Guinea pigs shows: Dark nucleus in the cells of zona reticularis-a, Light nucleus in the cells of zona reticularis-b, H&E stain, X400.



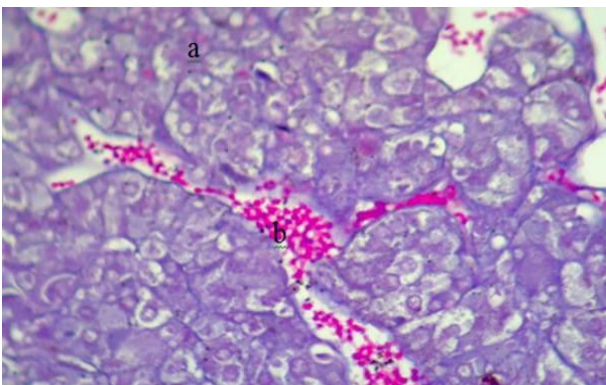
Figure,10: Zona reticularis of adrenal gland in Guinea pigs shows: Blood sinusoid-a, Connective tissue (septa)-b, Masson's Trichrome stain, X400.



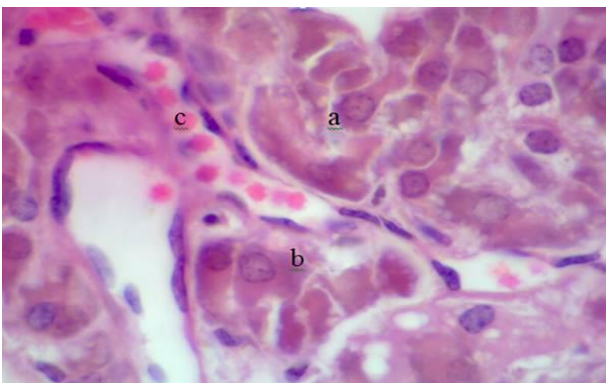
Figure,11: Medulla of adrenal gland in Guinea pigs shows: Zona reticularis-a, Chromaffine cells-b, Blood sinusoid-c, Central vein-d, H&E stain, X400.



Figure,14: Medulla of adrenal gland in guinea pigs shows: Epinephrine cell-a, Norepinephrine cell-b, Blood sinusoid-c, Potassium dichromate stain, X1000.



Figure,12: Medulla of adrenal gland in guinea pigs shows: Chromaffine cells-a, Blood sinusoid-b, Masson's Trichrom stain, X400.



Figure,13: Medulla of adrenal gland in guinea pigs shows: Epinephrine cell-a, Norepinephrine cell-b, Blood sinusoid-c, Potassium dichromate stain, X1000.

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دراسة شكلية-نسجية و كيمياء-نسجية للغدة الكظرية في ذكور خنازير غينيا البالغة

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الخلاصة

أجريت الدراسة الحالية علي عشرين ذكرا بالغ من خنازير غينيا في قسم التشريح والأنسجة لكلية الطب البيطري جامعة بغداد، لغرض تمييز الصفات الشكلية- نسجية والكيمياء- نسجية للغدة الكظرية. شكليا، خنازير غينيا تمتلك زوج من الغدد الكظرية بنية اللون تقع انسيا اماميا متصلة الي كل قطب امامي للكلى، الغدة الكظرية اليمنى تمتلك شكلا كمثري بينما اليسرى تمتلك شكلا طوليا. اظهرت الدراسة الاحصائية وجود فرق معنوي بين الغدة الكظرية اليمنى واليسرى في الوزن، الطول، السمك، العرض و الحجم. اظهرت الدراسة النسجية والكيمياء نسجية بان الغدة الكظرية تتكون من القشرة واللب. القشرة تتكون من ثلاث مناطق، المنطقة الحبيبية تتكون من خلايا عمودية او مكعبة مرتبة في مجاميع مثل الكبيبية، المنطقة الحزمية والشبكية تتكون من خلايا مضلعة تترتب في حبال منتظمة وغير منتظمة. منطقة اللب تتكون من مجاميع بيضوية من الخلايا (خلايا كرمافين) التي تترتب في حبال غير منتظمة مفصولة بواسطة جيبانبات دموية ومحاطة بواسطة وريد مركزي، تحتوي نوعين من الخلايا، الخلية الاولى عمودية الشكل بنية اللون تمثل الخلية الفارزة للابنفرين، الخلية الثانية مضلعة الشكل ذات لون بني فاتح و نواة كروية تمثل الخلية الفارزة للنورابينفرين عند تثبيتها في املاح الكرومات. اظهرت جميع اجزاء الغدة الكظرية تفاعل قوي الي معتدل للصبغة حامض شف. اظهرت النتائج الاحصائية وجود فرق معنوي بين الغدة الكظرية اليمنى واليسرى في سمك المحفظة، المنطقة الحبيبية، المنطقة الحزمية، المنطقة الشبكية ومنطقة اللب..

الكلمات المفتاحية: النسجية، الكيمياء النسجية، الغدة الدرقية، خنازير غينيا.