MODERN MEDICINE AND PRACTICE
https://inovatus.es/index.php/ejmmp

EUROPEAN JOURNAL OF MODERN MEDICINE AND PRACTICE
Vol. 4 No. 6 (Jun - 2024) EJMMP ISSN: 2795-921X

DESCRIPTION OF THE ADRENAL GLAND HISTOCHEMISTRY, BIOCHEMISTRY AND PHYSIOLOGY IN RABBIT ORYCTOLAGUS CUNICULUS

Mohammed H. Hindi, Karrar K.Atyah
Lecturer Assistant, Department of Anesthesia, College of health and Medical Technology, Sawa University, Almuthana, Iraq

Ali A. Abd Al Hassan, Anhar Ahmed khinfor
Lecturer Assistant, Department of Medical Laboratories, College of health and Medical Technology, Sawa University, Almuthana, Iraq

Abstract: Objective: This study was intended to give insight on the histochemical, biochemical, and physiological characteristics of an adult rabbit's adrenal gland. **Materials and methods:** The study was conducted on nine animals. Adrenal tissue samples were obtained from various locations of the gland, fixed with formalin, and processed for light microscopy. **Results:** The current investigation indicated that adrenal glands are surrounded by a big, well-developed capsule and an adrenal cortex. This cortex is divided into three zones: glomerulosa, fasciculate, and reticularis. The glomerulosa was a layer directly beneath the capsules. This zone's cells are big and prominent, with huge nuclei that form columnar cords supported by many capillaries, fasciculate was largest zone in the adrenal cortex, made up of long straight cords of enormous polyhydral secretory cells, reticularis was cortex's deepest layer, made up of an extremely brief anastomosing network of secretory cell cords separated by sinusoids. These cells tend to be more deeply stained than other zones. The medulla was composed of large pale-staining hexagonal cells organized in little anastomosing strings secured by reticular fiber; these cells have been separated by sinusoids which intervene in adjacent cords; and autonomic ganglionic cells, that are large cells that can be noticed alone or in groups. In medulla, epinephrine secreting cells were large spherical cells with light acidic contents and ovoid enormous prominent dark nuclei with numerous nucleoli. The smaller cells with brownish nuclei are nor epinephrine cells, which were incorporated in into the previous cells, sinusoidal capillaries, the adrenal medulla secretes a number of hormones, Epinephrine and norepinephrine -secreting cells were response increases heart rate and respiratory rate performance, while also stimulating primordial cells of capsule and glomerulosa cells, resulting in the body's homeostasis being adjusted and efficient. **Conclusion:** The study found that the rabbit had an efficient developed adrenal gland. Both cortex and medulla exhibit cell divisions.

Key Words: Histochemical, physiology, adrenal gland, rabbit, epinephrine.

INTRODUCTION

Animals from laboratories are highly valuable animals used in scientific research and medical testing. Rabbits are classified as fermenters for nutrients which are consumed in hindgut, based on the bacteria present (1-3). The adrenal glands are endocrine glands that generate several hormones; aldosterone

adrenaline, and cortisol, regulates the body's sodium and potassium ions (the water and electrolyte balance), while cortisol affects the metabolic activity of protein, carbohydrates, and lipids (4-6). There is located above the kidneys. Each gland has an outer cortex and an inner medulla; however, their basic distribution differs amongst mammals. The adrenal cortex is divided into three subzones: zona glomerulosa, zona fasciculata, and zona reticularis.The adrenal cortex makes three types of hormones such as; glucocorticoids, mineralocorticoids, and androgens. The zona glomerulosa produces mineralocorticoids (such as aldosterone), which aid in pressure and electrolyte management (7,8). The zona fasciculata synthesizes glucocorticoids cortisol and corticosterone, which regulate metabolism and suppress the immune system. The zona reticularis, the cortex's innermost layer, produces androgens, which are transformed into fully operational sexual hormones in the the gonad and other target organs (9,10). Steroidogenesis is the process by which cortical cells produce steroid hormones (11). The adrenal cortex produces two types of hormones; glucocorticoids and mineralocorticoids. Mineralocorticoids; aldosterone and deoxycorticosterone) play an important role in maintaining the body's water and electrolyte balance, which is controlled by the renin-angiotensin system (12, 13). It has been stated during normal function, the adrenal medulla secretes a small number of hormones, however during an acute stressed reaction, it secretes a significant amount (14). Epinephrine-secreting cells are larger than norepinephrine-secreting cells, with diameters more than twice that of norepinephrine; instead, epinephrine is very large to animal adapt to the fight or flight reaction. This response increases heart rate and respiratory rate performance while also stimulating primordial cells of capsule and glomerulosa cells, resulting in the body's homeostasis being adjusted and efficient (15, 16). There has been very little research published on the features and biochemical tests of the adrenal glands. The goal of this study was to provide light on adrenal gland structure and function of it by histochemical and biochemical methods.

Materials and Methods

Ethical approval

The procedures were performed in the compliance with animal care, Iraq; during January 2024.

Study animals: This study used nine healthy adult rabbits, *Oryctolagus cuniculus*, their age 7-9 months; who are nonruminant herbivores that prefer low fiber plant sources, were bought from mark in Al-Muthanna City.

Samples collection: The adrenal glands were collected immediately following anesthesia with a ketamine and xylazine overdose then the animals were sacrificed (17), washed with distilled water, and placed in a plastic container with a buffer formalin for 24 hours before being transported to the laboratory for routine paraffin section processing.

Histological study: Samples were taken from various areas of the adrenal gland, dehydrated using successive spiral of ethyl alcohol, cleaned by xylen, infiltrated and embedded in paraffin wax, and cut by rotary microtome (18).

Tissue staining: The stains that used; hematoxylin and eosin, Masson's trichrome (18).

Histochemical staining:

Periodic acid schiff (PAS) was used to treat mucopolysaccharides, , glycoproteins, , and basement membrane. Sections were immersed in a water-based solution of periodic acid for a period of thirty minutes. The sections were then washed to eliminate any acid residue, followed by Schiff's reagent for 30 minutes and potassium metabisulfite 0.55% for 1 minute (18).

Alcian blue pH 2.5: The process for acidic mucopolysaccharides using alcian blue at pH 2.5 is as follows: Deparaffinize using xylene, rehydrate using graduated ethanol, alcian blue on thirty minutes, water from the

faucet for five, then nuclear fast red for a total of ten minutes. Water for a minute. Dehydrate with a gradient of ethanol (18).

STATISTICAL ANALYSIS:

This study is analyzed by using the one-ways analysis of variance (ANOVA) test at a 1% level of significance. Data were processed and analyzed by using statistical program social science (SPSS).Thickness of cortex and medulls. Mean (x-) ± standard error (S E) calculated to each the measurements (19).

RESULTS AND DISCUSSION:

The adrenal glands were a pair of bean-shaped glands that stand on top of the kidneys and reside in the back abdomen, between the extra midline kidney and the diaphragm. They were hard, They were surrounded by a large, well-developed capsule; contains dense, uneven connective tissue and, on occasion, smooth muscle fibers. The capsule has narrow trabeculae that pass into the cortex but less into the medulla (Fig. 1-4). This was corroborated by (20, 21). The capsule was composed of two layers of dense connective tissue: an exterior layer and an interior layer containing smooth muscle cells (Fig. 1). This is comparable to the findings of (22) in porcupine. It was found that the regrowth and substitution of damaged and dead adrenal cortex tissues occurs via compensation by undifferentiated primitive cortical cells. The physical adaptability in its structure, which meant the size of their organs, particularly adrenals, was reduced despite their efficiency, which was to flee from predatory animals. This is corroborated by (5,23). The inner layer contained smooth muscle cells. Smooth muscle cells stimulate cellular secretion by contracting and applying pressure to the cells. The groups of undifferentiated cells found in the capsule were thought to serve as a reservoir for the underlying zones, as validated by (24). The adrenal cortex; consists of parenchymal cells lies between the capsule and the medulla. This cortex comprises the following areas: area glomerulosa, area fasciculate, and area reticularis (Fig.5-8), this similar to (25) in rabbit. The glomerulosa lies right under the gland's fibrous capsule (Fig. 6). This aligns with (5, 26). The cells in this zone release mineral corticoid hormone, which regulates the body's Na+ and K+ ions, as well as the balance of water and electrolytes (6). There is a clear line of distinction between two regions, indicating as there is no cellular transfer between them. In contrast, the absence of a clear line separation shows cellular interaction between the two layers. These areas were composed of two spherical cellular clusters, The cells in the area were large and visible, with massive nuclei and columnar cords surrounded by many capillaries (Fig. 3).

The reticularis comprised the deepest layer, made up of a short anastomosing network of secretion cell cords separated by sinusoids. These particular cells were frequently more deeply stained than other zones. Its small cells form irregular cords and clusters, separated by capillaries and connective tissue (Fig.4), The zona fasciculate was the biggest of the three layers of the cortex, consisting of long straight cords of massive polyhydral secretory cells located between the zona glomerulosa and zona reticularis. The cells are grouped in columns radially orientated towards the medulla (Fig. 5). The cells of this zone, along with the zona fasciculate, secrete glucocorticoid hormone, which operates on the metabolic activity of carbohydrates, proteins, and fat (3,26). The zones of cortex had a mutual interaction. There was no clear dividing line between the zones, zona fasciculata was greatly developed due to the thinness of the zona reticularis. This was demonstrated by the lack of a line of demarcation among the layers. This is consistent with (25), who reported that the distinction among zona fasiculata and zona reticularis in goats was absent. The configuration of cells in this area as glomeruli was consistent with the findings of (6), who discovered that the cells in this zone were structured as glomeruli. This layout differed from horse, canine, and pig cells, which are organized in arcs with convexity oriented toward the periphery (15).The thicker the zona glomerulosa, the more aldosterone is secreted, which affects the distal tubules of the kidney, increasing salt absorption and renal efficiency during fight or flight and maintaining body homeostasis. This explains the occurrence of an outerdark acidophilic section that symbolizes active stimulated layer, as well as an interior

bright pale component that depicts the zone's resting condition. By contracting on the clusters, zona glomerulosa cells secrete more efficiently. This zone was the closest to the capsule and will therefore receive the greatest number of undifferentiated compensatory primordial cells (Fig.4).

The current study found that the organization of zona fasiculata cells into wide sheets indicates this area was most efficient area in the adrenal. This will obscure the parallel pattern of cell connections seen in other species. This was supported by the significant concentration of mitotic figures. This zone's spongy appearance implies that lipid droplets dissolved during standard histological preparation. This is in line with (7).The outside layer of fasiculata contained a high concentration of lipids, while the interior two-thirds contained just trace amounts of lipid. This is similar to (8).

Our findings indicated that outer fasiculata was the most active section of this zone due to blood vessel engorgement and higher mitotic figures. Fat depletion suggested hyperactivity in this zone. This finding seems to be consistent with (8). (9) reported that higher lipid levels in buffalo were associated with glucocorticoid release, and the presence of apoptotic cells all indicate that this layer was inactive and served as a graveyard for cortical cells in the adrenal cortex. This mirrored the migration theory, which recorded zona reticularis. This was consistent with (11). Who stated that cortical cells start in the cortex's outermost layers and migrate lower to die there.

The medulla was made up of large pale-staining conical cells arranged in tiny anastomosing links supported by reticular fibers, separated by sinusoids, as well as autonomic ganglionic cells, which are larger cells that can be found alone or in groups. Epinephrine-secreting cells in the adrenal gland's medulla were enormous cylindrical cells containing light acid cytoplasm and oval huge, conspicuous dark nuclei with multiple nucleoli. The little cells with darker nuclei include norepinephrine cells mixed up with the preceding cells, as well as sinusoidal capillaries. The dimensions and thickness of adrenal zones differ by species and gender. The configuration of epinephrine and norepinephrine releasing cells differed amongst animals. (Fig.7,8). Our findings revealed medullary cells are largely made up of epinephrine-secreting cells on the outside and norepinephrine-secreting cells inside (Fig.8). This is analogous to the discovery of (10) in guinea pig and contrasts with (12). Who claims that in porcupine , the adrenal medulla's periphery was norepinephrine area and the core part was epinephrine area. (13) Although the majority of medullary cells release epinephrine, the two types are scattered randomly throughout the medulla. Among herbivorous domestic species such as ruminants, horses, and pigs, chromaffin cells are largest in the outer section of the medulla and the lowest in the inner portion, which is consistent with the current study. The overlapping phases of chromaffin cells show that cellular materials are functionally exchanged between medullary cells. The number and size of epinephrine-secreting cells were greater than of nor epinephrinesecreting cells. This was similar to that in the dog and human, and in contrast with cats that secrete primarily nor epinephrine (10).

Epinephrine-secreting cells increase the body's metabolism and raise its temperature. Furthermore, glycogenolysis was boosted to increase glucose levels in the blood, which is used as an energy source. This was in line with (14). The many methods for preserving water temperature balanced in animals. High ambient temperatures react (15). The level of aldosterone rise following prolonged exposure to a hot environment and during dehydration (8). The glucocorticoids (hydrocortisone, Cortisone, cortisol, and corticosterone) are generated by the zona fasciculate and the zona reticularis, all of which collaborate in fat, protein, and carbohydrate metabolism and are regulated by ACTH from the pituitary gland, in reticularis were able to release mostly corticoids and create weak androgens. The adrenal cortex, viz., glucocorticoids, a large amount of energy, however mineralocorticoids keep water and salt balance in the body, and adrenal medulla that secrete adrenalin, which activates the heart muscle and rate, intensity, constriction, and can animals adapt with quick temperature changes, and noradrenaline, which participates in the maintenance of blood pressure and the activation of smooth muscle (9,10).

The stimulation of preganglionic autonomic nerve fibers is an element of the organism's protective response to stress, including as flight, terror, combat, cold, and pain. Acute heat exposure produced a strong stressful effect on the rat adrenal gland; the adrenal gland reduced under these conditions, as pointed to by (11). The cortex is lipid-rich and responsible for corticosteroid release.Undernutrition resulted in increased vacillations, increased cell size, and even cell rupture, and congested blood vessels. There was also an increase in lipid in all cortical zones, particularly in the glomerulosa and fasciculate, indicating adrenals were in a state of higher secretory activity (9). Aldosterone is vital for the permanent control of blood pressure (6). The adrenal medulla thus influences heart rate, energy and metabolism. The cells of medulla produce hormones. The adrenal medulla converts amino acid tyrosine to the catecholamines adrenaline, norepinephrine, and dopamine. Adrenaline and noradrenaline cause accelerated heart rate with blood pressure, narrowing of blood vessels in the skin and intestines, smooth muscle dilatation (bronchiole and capillary), and accelerated metabolism, each of which are associated with the fight-or-flight response. Nerve impulses induce the release of catecholamines, and catecholamine receptors are widely dispersed throughout the body (12).

Table (1): Measurement of thickness of capsule, cortex layers and medulla of adrenal gland in rabbit µm (X^{-} ± S.E)

Species / Part	Capsule	Cortex	Zona glomerulosa	Zona fasciculate	Zona reticularis	Medulla
Right gland	36.1±0.2 A	941.4±1.3 A	118.1±0.4 A	734.2±2.1A	82.4±0.1A	127.2±0.02 A
Left gland	34.6±0.3 B	935.1±1.2 B	114.2±0.3 B	711.4±2.5B	80.4±0.2B	122.5±0.03 B

The values column with capital letters in the same raw denote to the significant difference (P>0.01) whereas raw values with small letters denote to the nonsignificant differences (p<0.01).

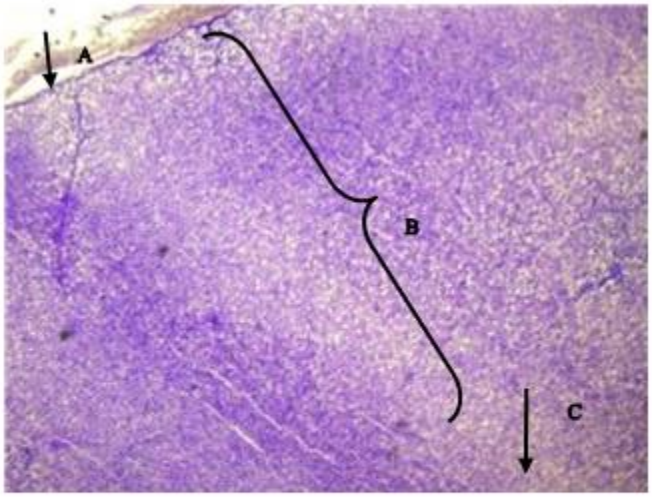


Fig.(1): Longitudinal section of rabbit adrenal gland ; capsule (A), cortex (B), medulla (C), H@E 100X.

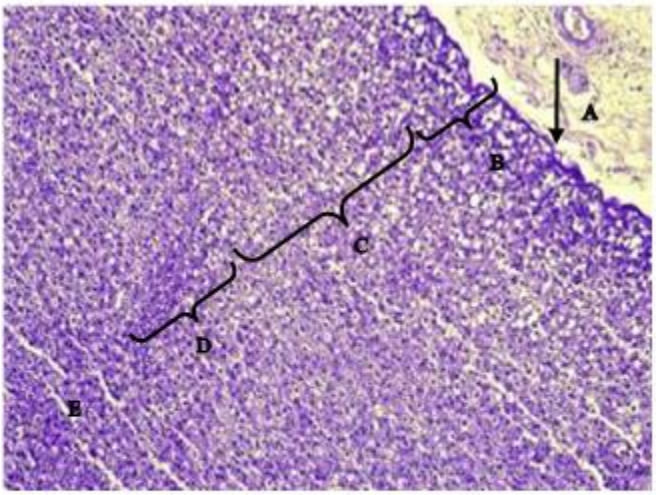


Fig.(2): Cross section of rabbit adrenal gland; capsule (A), glomerularis (B), fascularis (C), reticularis (D), medulla (E) ,PAS 100X.

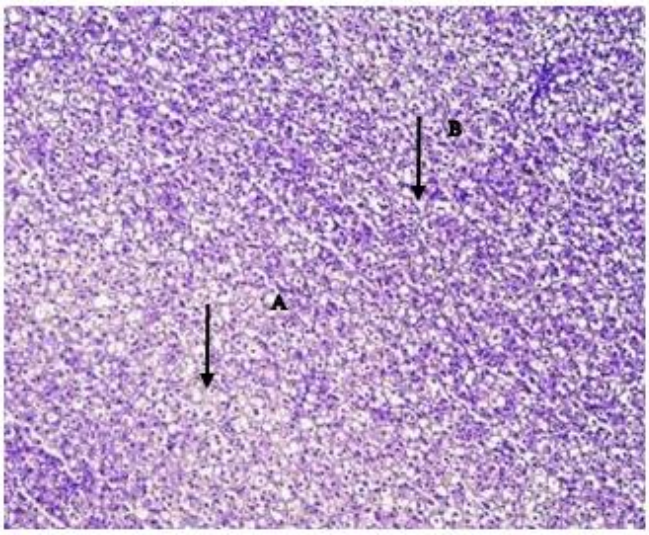


Fig. (3): Cross-section of rabbit adrenal gland; Cells of glomerulosa zona (A), cells of fasciculate (B), AB 400X.

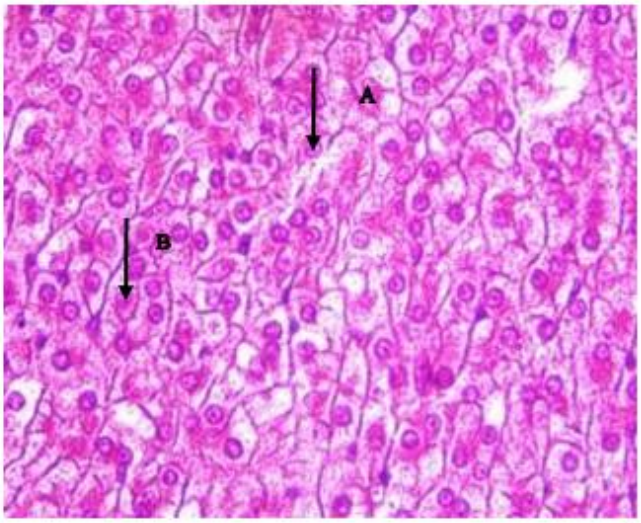


Fig. (4): Cross-section of rabbit adrenal medulla; nor-epinephrine cell (A), epinephrine cell (B), Masson 400X.

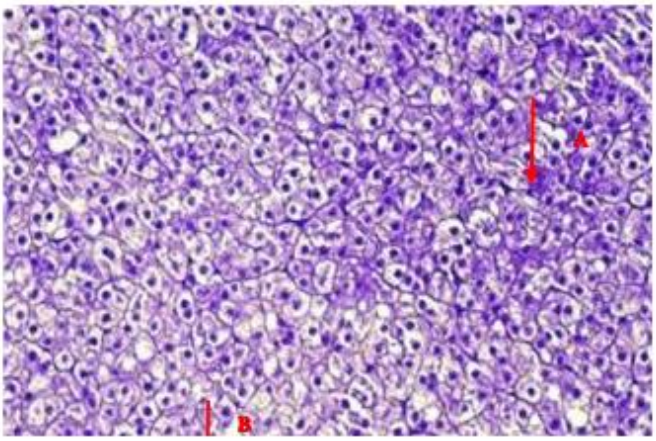


Fig. (5): Longitudinal section of rabbit adrenal gland; Cells of fasciculate zona (A), cells of reticularis (B), PAS-AB 400X.

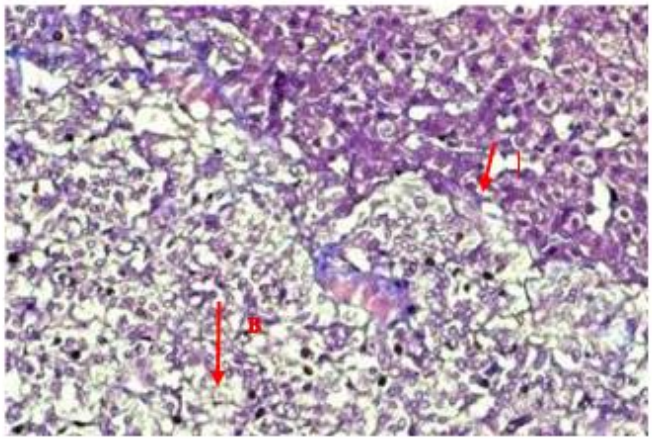


Fig. (6): Cross section of rabbit adrenal gland; Cells of reticularis zona (A), cells of medulla (B), Mallory 400X.

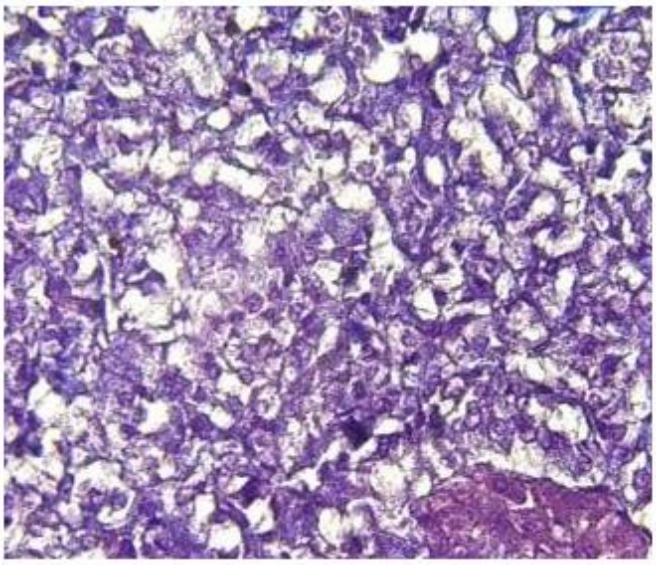


Fig. (7): Longitudinal section of adrenal medulla; nor-epinephrine cell (A), epinephrine cell (B), Masson, 400X.

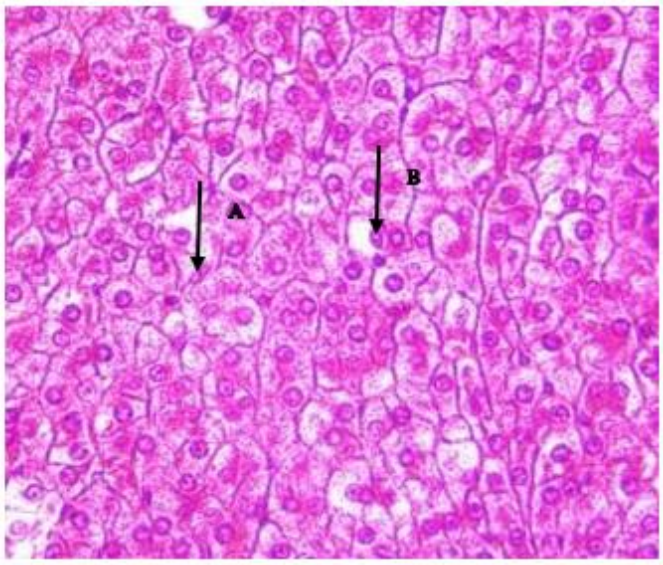


Fig. (8): Longitudinal section of adrenal medulla; norepinephrine cell (A), epinephrine cell (B), PAS, 400X.

Conclusion: The study found that the rabbit had an efficient developed adrenal gland. There is a distinct line of separation between two zones, indicating that there does not exist cellular interchange between them. On the other hand, no clear line demarcation indicates cellular exchange across the two levels. The zona glomerulosa consisted of two spherical cellular clusters.

Both cortex and medulla exhibit cell divisions..

Funding

This research did not receive external funding. All authors were contributing to supporting this work in a self-supporting manner

Availability of data and materials

Data of the current study area available with reasonable request.

Competing interest

There are no conflict of interest between the authors.

Ethical consideration

The current research has followed the accepted principles of ethical conduct by the college of veterinary medicine, Sawa-University, Iraq, during January 2024.

Author contributions

All authors contributed equally.

Acknowledgements

We extend our sincere appreciation to our dedicated team for their contribution this study, with special acknowledgment to Anatomy and histology department at veterinary medicine collage, AlMuthanna university, Iraq.

REFERENCES

1. Mapara M, Thomas BS, Bhat KM. (2012). Rabbit as an animal model for experimental research. Dent Res J. 2012;9(1):111-118. DOI: 10.4103/1735-3327.92960.
2. Struillou, X.; Boutigny, H.; Soueidan, A.; Layrolle, P. (2010). Experimental animal models in periodontology: A review. Open Dent. J., 4, 37–47.
3. Bosze Z, Houdebine LM. (2006). Application of rabbits in biomedical research: A review. World Rabbit Sci.;14:1-14. DOI: 10.4995/WRS.2006.712.
4. Batah, A.L.and Mirhish, S.M. Histomorphological and histochemical study of adrenal gland in adult male of guinea Pigs (*Cavia porcellus*). (2022). *The Iraqi Journal of Veterinary Medicine*, *43*(1), 59-66. https://doi.org/10.30539/iraqijvm.v43i1.475.
5. Suhett, W.G.; Gerez, J.; M.S. Hohmann, L. Staurengo-Ferrari, W.A. Verri, F.H.O. Pinho, L.D. de Barros, S.T. Cardim, K.M.C. Flaiban, Ana Paula F.R.L. (2023). Bracarense, Exploring porcine kidney explants as a model for the study of nephrotoxins and the therapeutic potential of phytic acid, Environmental Toxicology and Pharmacology,Volume 102,2023,104241,https://doi.org/10.1016/j.etap.104241.
6. Stieger-Vanegas SM and McKenzie E (2021). Imaging of the urinary and reproductive tract in small ruminants. Vet Clin Food Anim Pract. 2021;37(1):75-92. DOI: 10.1016/j.cvfa.2020.10.002.
7. Dutta P, Hakimi S, Layton AT. (2024). How the kidney regulates magnesium: a modelling study. R Soc Open Sci.; 11(3):231484. doi: 10.1098/rsos.231484.
8. Rossi, G.; Liu, K.-F.; Kershaw, H.; Riddell, D.; Hyndman, T.H.; Monks, D.; Musk, G.C. (2023). Biological Variation in Biochemistry Analytes in Laboratory Guinea Pigs (*Cavia porcellus*). *Vet. Sci.*, *10*, 621. https://doi.org/10.3390/vetsci10100621.
9. Yang, I.S.; Jang, I. and Yang J.O. (2023). CanISO: a database of genomic and transcriptomic variations in domestic dog (*Canis lupus familiaris*). *BMC Genomics* 24, 613. https: //doi. org/10.1186/s12864-023-09655-0.
10. Abass,T. A. (2017). Anatomical and histological study of adrenal gland in new natal and adult guinea pig (Caviaporcellus). *Kufa Journal for Veterinary Medical Sciences*, *8*(1):181-192.
11. Ekele, I; Uchenna, N and Ibe C.S. (2014). The Kidney and adrenal gland of the African Palm Squirrel *(Epixerus ebii*): A Microanatomical Observation.Rev. Fac. Cs. Vets. 55(2):60-67.
12. Sarwar, G; Sughar, P. and Aslam, M. S. (2008). Comparison of the effects of experimentally induced hypothyroidism in adrenal gland morphology in pre- postnatally treated albino rats .Pakistan Journal of Pharmacology. 25 (1):13-18.
13. Yılmaz, S and Girgin, A.(2005). Light and electron microscopic observations on the structure of the porcupine (*Hystrix cristata*) adrenal gland*Vet*. arhiv*75*(3): 265-272.
14. Enemali, F.U. (2014).Anatomical Studies on Thyroid, Parathyroid and Adrenal gland of The African Giant Rat(Cricetomys gambianus Water House-1849),M.Sc, Thesis,Faculty of medicine,Ahmudu Bello University,Zaria,Nigeria.
15. Dyce KM, Sack WO, and Wensing CJG (2010). Textbook of veterinary anatomy.4rd ed. W. B. Saunders company. Philadelphia, Pp: 554 - 694.
16. Bacha, W. J and Bacha, L. M. (2000).Color Atlas of Veterinary Histology. Second Edition. *Lippincott Williams & Wilkins. AWolters Kluwer Company.* PP:191 – 200.

17. Mahmood MB. (2022). A comparison between ketamine-xylazine and ketamine-midazolam or all of them to induce balance anesthesia in rabbits. Iraqi J Vet Sci.; 36(2): 499-506. DOI:10.33899/ijvs.2021.130618.1852.
18. Suvarna SK, Layton C, and Bancroft J D (2018). Bancroft's theory and practice of histological techniques, 8th ed. Churchill Livingstone Elsevier Philadelphia, Pp: 176 -725.
19. Al-Rawi KM and Kalaf-Allah IS (1980). Design and Analysis Agriculture Experiments. Dar-Al Kutub-Mosul, Iraq, Pp: 65, 95-107.
20. Abass,T. A. (2017). Anatomical and histological study of adrenal gland in new natal and adult guinea pig (Caviaporcellus). *Kufa Journal for Veterinary Medical Sciences, 8*(1):181192.
21. Sarwar, G; Sughar, P. and Aslam, M. S. (2008). Comparison of the effects of experimentally induced hypothyroidism in adrenal gland morphology in pre- postnatally treated albino rats .Pakistan Journal of Pharmacology. 25 (1):13-18.
22. Yılmaz, S and Girgin, A.(2005). Light and electron microscopic observations on the structure of the porcupine(*Hystrix cristata*) adrenal gland*Vet*. arhiv*75*(3): 265-272.
23. Sheikhian, A; Saadatfar, Z andMohammadpour, A. A. (2014). A histological study of adrenal gland in guinea pig and hamster, Comp Clin Pathol.111:782-789.
24. Silva, E. J; Felicio, L.F; Nasello, A. G; Zaidan-Dagli, M. and Anselmo-Franci J.A. (2004). Prolactin induces adrenal hypertrophy. Brazilian Journal of Medical and Biological Research, 37:193-199.
25. Hussein, A. A. ., & Abass, T. A. . (2015). comparative anatomical and histological study of the rabbits (oryctolagus cuniculus) adrenal gland at thirty and ninety days AGE. *Diyala Agricultural Sciences Journal* , *7*(2), 1–11. Retrieved from https://journal.djas.uodiyala.edu.iq/index.php/dasj/article/view/1572.
26. Mahshid Johnny, Nader Goodarzi, Mohsen Akbari Bazm (2022) Histological, transmission electron microscopic, and immunohistochemical study of the adrenal gland in the Persian squirrel (*Sciurus anomalus*). Microscopy Research and Technique, 85(2), 738.10.1002/jemt.23945.