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ORIGINAL ARTICLE

A histological study of adrenal gland in guinea pig and hamster

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Abstract In basic sciences, laboratory animals have a special role in investigations. In this study, the adrenal of three adult female guinea pigs and hamsters were studied. The left adrenal was larger than the right in two species. In hamster, it was in the cranial pole, and in guinea pig, it was in the craniomedial pole of the kidney. In guinea pig, the right and left glands were bean shape and in hamster, egg shape. This gland was covered by a capsule without muscle fibers in guinea pig and hamster. The peripheral cortex in hamster was divided into three zones and in guinea pig, four zones. Zona glomerulosa was composed of arcuate cords in guinea pig and clusters in hamster. The foamy cytoplasm of fasciculate in guinea pig was more than hamster. There was also a lot of lipofuscin in cytoplasm of reticularis in the two animals, especially in guinea pig. The medulla in guinea pig was long and in hamster was egg-shaped. In guinea pig, reticularis cells penetrated into medulla and invagination of cortex also was obvious in medulla of this species. In chromaffin reaction, the less electron-dense granules were epinephrine and the dense granules were norepinephrine cells.

Keyword Histology · Adrenal · Guinea pig · Hamster

Introduction

The adrenal glands are one of the most important glands in the body; in mammals, it is generally made of two layers: outer cortex and inner medulla. They have different origins during embryogenesis. The adrenal cortex derives from the mesoderm and the adrenal medulla from the neural crest (Karpac et al. 2005), but they merge to a single organ during embryological development. The two parts differ from microscopic structure and function. The adrenal cortex is subdivided into three or four zones of epithelial cells (Dellmann 1993) (Fig. 1). The zona glomerulosa is the outermost zone. It may form irregular clusters or cords in human and bovids, or arranged into arcs so as in carnivore, horse, and pig (Banks 1993). Zona intermedia consist of small closely packed cells between the zona glomerulosa and zona fasciculata. This zone is seen in some domestic mammals as carnivores and horse. The zona fasciculata is the widest zone of cortex. It is formed by radially arranged cords of polyhedral or cuboidal cells. For presence of numerous lipid vacuoles in their cytoplasm, they appear as foamy cells. The innermost zone of adrenal cortex is zona reticularis. It is arranged as an irregular network of anastomosing cords. The cells are roughly the same in morphological features as fasciculata but their nuclei and cytoplasm have darker staining (Banks 1993). The adrenal medulla is composed of chromaffin cells which form clusters and anastomosing cords. There are two types of chromaffin cells in adrenal medulla. The norepinephrine cells contain a large spherical nucleus and highly electron-dense granules. The epinephrine cells are similar to norepinephrine cells but their granules are less electron dense and there is a small empty space between granules and boundary membrane (Dellmann 1993; Steven and Lowe 1991). Rodents are one of the largest orders of mammals. Most people are familiar with hamsters and guinea pigs, which are kept as pets. Despite the great species diversity, they have shared common features. Most rodents are herbivorous but some are omnivorous and some prey on insects. The guinea pig (*Cavia porcellus*), belongs to the family Caviidae and the genus *Cavia*. Biological experimentation on guinea pigs has been carried out since the

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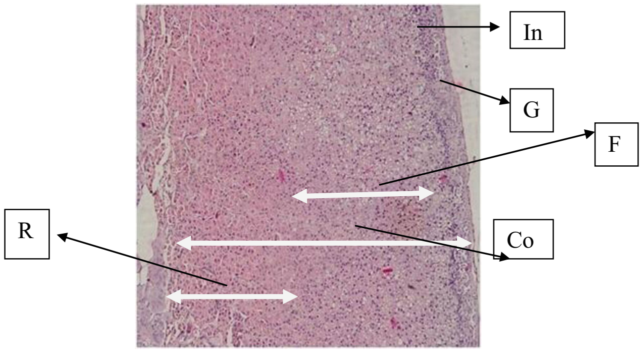

Fig. 1 The cortex (C) in guinea pig. Zona glomerulosa (G), zona intermedia (In), zona fasciculate (F), and zona reticularis (R). (H&E, ×320)

seventeenth century and it is used as a test subject in the nineteenth and twentieth centuries. Grass is the guinea pig's natural diet. The Syrian hamster or golden hamster was first described scientifically in 1839 (Bahadori et al. 2009). It belongs to the family Cricetidae and superfamily Muroidea. Their diets include a variety of foods including dried food, berries, nuts, fresh fruits, and vegetables, so hamsters are omnivores. Because of sex differences in adrenal structure, this study was on the structure of adult female guinea pig and hamster.

Materials and methods

In this study, three adult female guinea pigs and hamsters were separated and sacrificed under ether anesthesia. The abdominal cavity was opened and 12 adrenal glands were freed from adjacent tissues and weighed individually. Samples were fixed with 10 % formaldehyde and also Zenker formalin. After

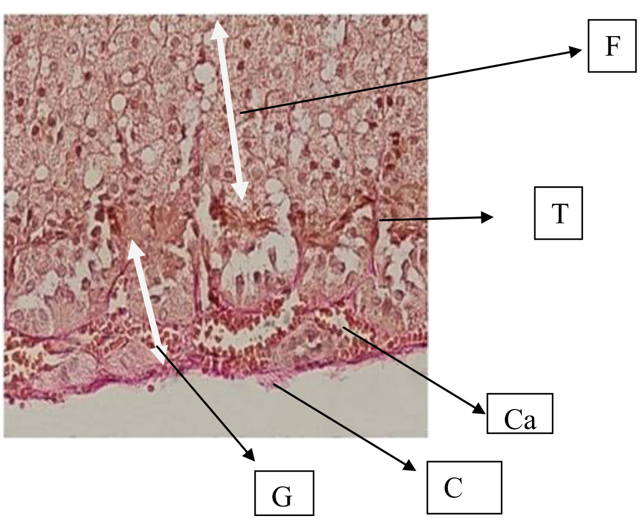

Fig. 2 The zona glomerulosa and fasciculata in guinea pig. Zona glomerulosa (G), zona fasciculate (F), capsule (C), capillary (Ca), and trabecula (T). (Van Gisson, ×640)

Table 1 Measured parameters in adrenal gland of guinea pig (μm)

Parameters	Mean±SD	Max	Min
Cortex (width)	1203±68/484	1350	1100
Zona glo (width)	123±10/593	150	110
Zona fa (width)	632±25/298	650	590
Zona ret (width)	448±58/462	590	380
Total large diameter	6342/5±747/243	7125	5375
Total small diameter	2888/5±355/912	3275	2375
Large diameter of medulla	3255±111/679	3375	3000
Small diameter of medulla	650±33/333	700	600

fixation, they were treated with ethyl and xylol and embedded in paraffin blocks. Sections (6-μm thick) were made by microtome and stained with hematoxylin eosin (H&E). The Mallory stain and Van Gisson staining method were used to demonstrate collagen fibers. For observation of the chromaffin reaction of medullary cells, the tissues were fixed in Zenker formalin. Then, after processing, they mounted on slides to demonstrate chromaffin cells. Morphometric evaluation on slides carried out with ocular micrometer and images were taken with a camera (Nikon) attached to a microscope.

Results

Anatomical structure

The adrenal of guinea pig was bean-shaped and located in medial of cranial pole of kidney; and in adult female, the average weight, length, width and thickness in right gland was 0.35±0.05 g, 10.6±1, 4.5±0.06, and 3.5±0.21 mm and it was 0.4±0.05 g, 11.2±0.76, 4.8±0.29, and 3.8±0.49 mm in left adrenal gland, respectively. The adrenal gland of adult female hamster was in oval to egg-shaped and located at the cranial pole of the kidneys. The average weight, length, width, and thickness were 0.03±0.18 g, 5±0.12, 3±0.21 and 2± 0.15 mm in right and 0.05±0.18 g, 5.3±0.29, 3.5±0.20, and 2±0.20 mm in left gland.

Table 2 Measured parameters in adrenal gland of hamster (μm)

Parameters	Mean±SD	Max	Min
Cortex (width)	669±28/848	700	610
Zona glo (width)	69±3/162	70	60
Zona fa (width)	405±8/498	420	390
Zona ret (width)	195±21,213	220	160
Total large diameter	2242/5±67/752	2350	2175
Total small diameter	1700±55/277	1775	1650
Large diameter of medulla	967±84/204	1125	875
Small diameter of medulla	1700±44/214	775	625

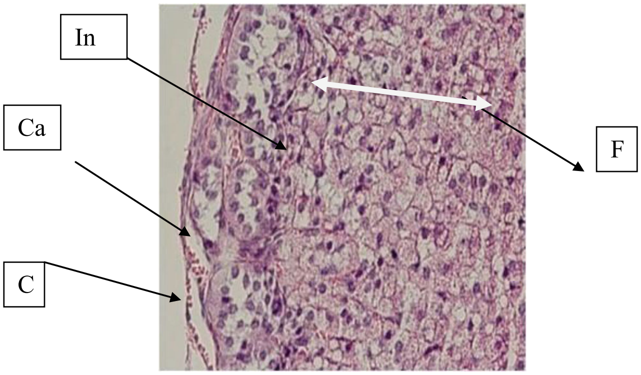

Fig. 3 The zona glomerulosa and fasciculata in guinea pig. Zona glomerulosa (G), zona intermedia (In), zona fasciculate (F), capsule (C), and capillary (Ca). (H&E, ×640)

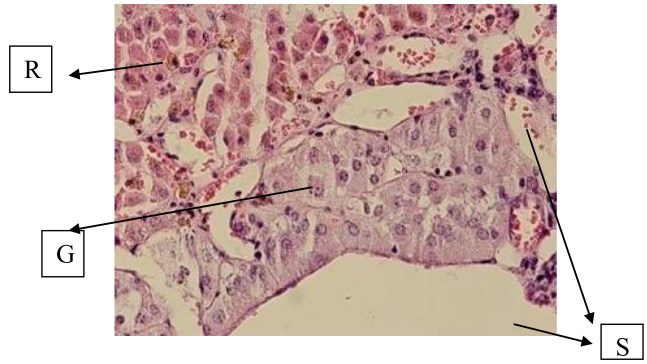

Fig. 5 A part of zona reticularis and medulla in guinea pig. Reticularis with lipofuscin pigments (R), sinusoid (S), and ganglion cell (G). (H&E, ×640)

Histological structure

The surface of hamster and guinea pig adrenal glands were covered by a connective tissue capsule; which in hamster, was very thin and in guinea pig, had a network of blood vessels (Fig. 2). There were no muscle fibers in capsule of adrenal. The connective tissue of capsule penetrated into the parenchyma and formed scattered collagen fibers in parenchyma. The adrenal cortex was subdivided to zones, each with different cell arrangement. The thickness of cortex was 1023±68.48 μm in guinea pig and 669±28.84 μm in hamster (Tables 1 and 2). Below the capsule, there was formed zona glomerulosa. It was very thin (the mean thickness in guinea pig 123±10.59 μm and in hamster 69±3.16 μm) and composed of cuboidal to columnar cells in guinea pig and cuboidal to short columnar cells in hamster. This zone was arranged in arches with the convex side facing the capsule in guinea pig (Fig. 3) and clusters in hamster. The cells had large dark nuclei. Blood capillaries could be found between the arches in guinea pig and hamster (Fig. 2). The zona fasciculata was 632±25.29-μm thick in guinea pig and 405±8.49 μm in hamster (Tables 1 and 2). The cells of this zone contained radially oriented cords of cells with one or two cell rows in each cord. There were blood capillaries between cords. In guinea pig, the cells were polygonal-shaped with foamy cytoplasm because of lipid vacuoles (Figs. 1, 2, and 3). In hamster, this area had cuboidal cells with small vacuoles in the outer cells of this zone. In guinea pig, between the zona arcuata and fasciculate, there was a thin layer of small cells with irregular, dense nuclei (Figs. 1 and 3). This zone can be represented as intermediate zone. The deepest layer of the adrenal cortex was zona reticularis. This zone was 448±58.46-μm thick in guinea pig and 195±21.21 μm in hamster (Tables 1 and 2). The zona reticularis appeared as irregular cords and was smaller than the other two zones of cortex. In guinea pig, the cells were polygonal with eosinophilic cytoplasm. There was also a lot of lipofuscin in cytoplasm (Figs. 4 and 5). Between cells, capillaries often appeared dilated. In hamster, the cells of this zone had relative basophilic cytoplasm with lipofuscin and often binucleate cells could be seen (Figs. 6, 7 and 8).

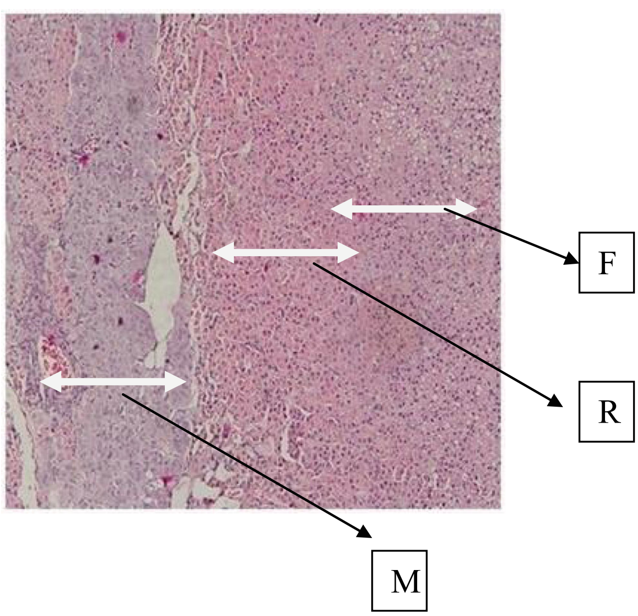

Fig. 4 The zona fasciculate, reticularis, and a part of medulla in guinea pig. Zona fasciculate (F), zona reticularis (R), medula (M). (H&E, ×320)

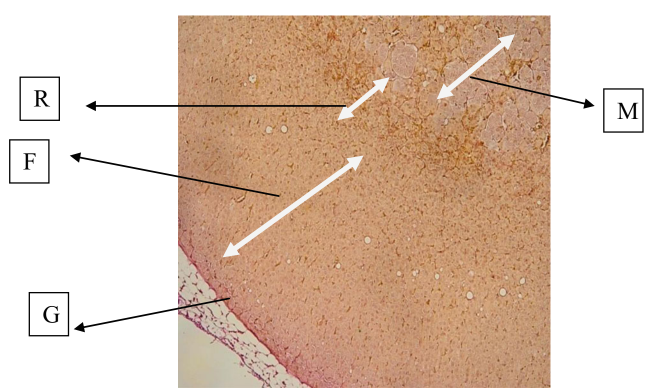

Fig. 6 Cortex and medulla in hamster. Zona glomerulosa (G), zona fasciculate (F), zona reticularis (R), and medula (M). (Van Gisson, ×160)

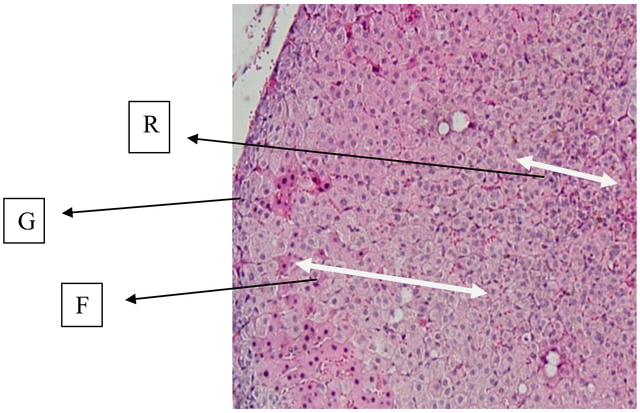

Fig. 7 Cortex in hamster. Zona glomerulosa (G), zona fasciculate (F), and zona reticularis (R). (H&E, ×160)

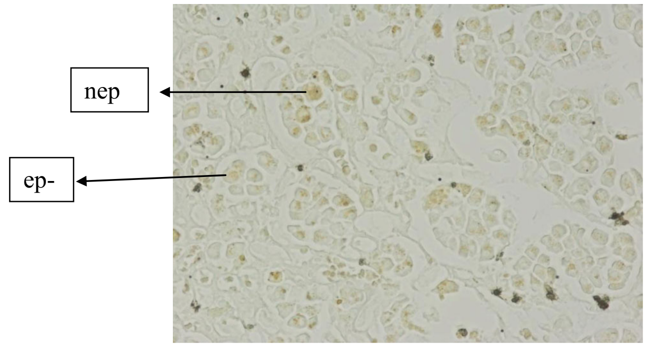

Fig. 9 Chromaffin reaction of cells in medulla of guinea pig. Epinephrine cells (ep), Norepinephrine cells (nep). (×640)

The medulla had a well-defined border with cortex. In guinea pig, invaginations of cortex tissue into the medulla were noticed. Medullary cells were larger and most of them, in both, had polygonal shape and an oval light nucleus which is eccentrically located. The cells showed a basophilic cytoplasm (Figs. 5 and 8) in adrenal gland of guinea pig, numerous sinusoidal vessels was present in medulla (Fig. 5), and in hamster, a large central venule was noticed in place (Fig. 8). The chromaffin cells in irregular clusters were observed. Some of them had less electron-dense granules with an empty boundary, but some had highly electron-dense granules (Fig. 9 and 10). Ganglion cells also were observed in medulla.

Discussion

In this study, the structure of adrenal glands in female guinea pig and hamster were studied. The form of this gland was different; in guinea pig, it was bean-shaped and in hamster, it was egg-shaped. In rabbit, the right adrenal is leaf-shaped and the left is bean-shaped (Parchami and Fatahian 2011).

In cow and dog, also the left and right glands have different form; in cow, the right is V-shaped and the left is C-shaped. In dog, the right is triangular and the left is wide. In sheep, two glands are bean shape.

From the view of position to kidney, in guinea pig, it was in craniomedial pole of kidney and in hamster, it was at the upper or cranial tip of kidney. In rabbit, it is separate from kidney and lay near the blood vessels. In two species, the average weight, long and wide of left, was more than right. From this view, it is similar to many other animals and humans (Nabipour et al. 2008).

On the adrenal gland of guinea pig, there was a capsule with many blood vessels and in hamster, it had a very thin capsule. In the two, between capsule and zona glomerulosa was an obvious border that's similar to horse, cat, rabbit, mouse, and rat (Cater and Lever 1954).

The adrenal cortex of mammals, as described, was built of three or four zones: the zona glomerulosa, zona fasciculate, and zona reticularis (Bragulla et al. 2004; Clark et al. 2005,

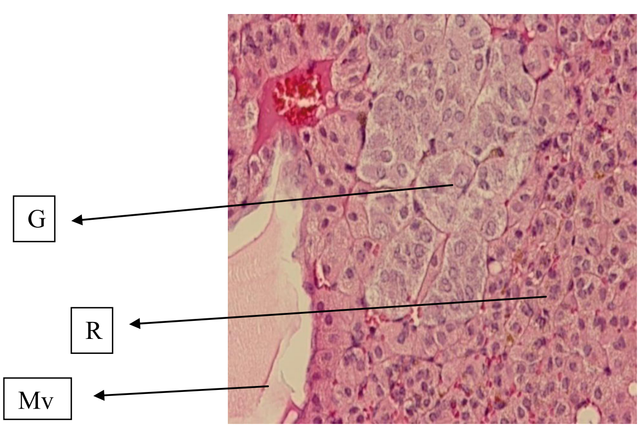

Fig. 8 A part of zona reticularis and medulla in hamster. Reticularis (R). ganglion cell (G), medullar vein (Mv). (H&E, ×640)

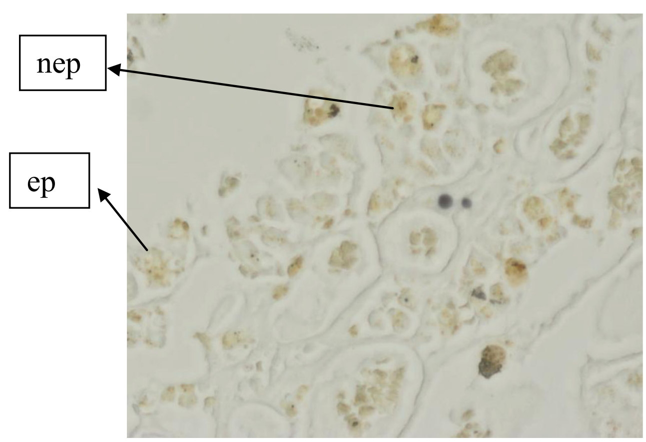

Fig. 10 Chromaffin reaction of cells in medulla of hamster. Epinephrine cells (ep), Norepinephrine cells (nep). (×640)

2008). It may also be an intermediate zone between glomerulosa and fasciculata. Zona glomerulosa is the outermost zone and is just beneath the capsule; it secretes mineralocorticoids and contains prismatic cells that formed arcuate cords in guinea pig. It is like dog and horse. In hamster, it had cuboidal and polygonal cells that formed clusters and similar to ruminants, it can be named glomerulosa. The mean thickness of the zona glomerulosa in guinea pig was 123± 10.59 μm and contained 10.22 % of cortex; in hamster, it was 69±3.16 μm and contained 10.3 % of cortex. In guinea pig, a thin layer of small cells was interposed between glomerulosa and fasciculate that the number of cells per unit was great. The intermediate zone constitutes a region in some domestic mammals as horse and carnivore (Banks 1993).

The fasciculata is the broadest zone, consists of radial cords with foamy cells, and has more lipid vacuoles to other zones. It was 25 % of cortex in guinea pig and 61 % of cortex in hamster. The cells of this zone were cuboidal in hamster and polygonal with more vacuoles in guinea pig. The vacuoles caused by numerous empty spaces from dissolution of lipid droplets during processing that described for many domestic animals (Dellmann 1993) and also in humans, but in some animals, for example in the bottlenose dolphin and striped dolphin, was not observed (Vukovic et al. 2009). Zona fasciculate in human forms 78/4 % of cortex, in dog and rabbit 60 % of cortex (Tanyolac 1993), and it consists 66/3 % of cortex in porcupine (Yılmazs and Girgin 2005).

Zona reticularis is the innermost zone and is next to the medulla. The cells appeared as irregular cords and are smaller than other zones. It occupied 37 % of cortex in guinea pig and 29 % in hamster. In human, it is 6/4 % and in dog 15 % (Sisson and Groossman 1975). Some previous studies stated the sex difference in size of reticularis; so in female hamster, it is smaller than male (Nikicicz et al. 1984). The cells of this zone arranged as irregular anastomosing cords that surrounded by sinusoids and cytoplasm of this zone had fewer vacuoles, it showed basophilia in guinea pig and eosinophilia in hamster. In the two, there were lipofuscin pigments, especially in guinea pig. In most other species, they also showed these pigments and some researchers believe that the cells of this layer have a degeneration process. It must be mentioned, according to previous studies, rodents have no functional zona reticularis and they are devoid of secretion the androgens (Keegan and Hammer 2002). In some studies in rodents, this zone, named as X-zone, and the function of it, is unclear (Hershkovitz et al. 2007).

In shape, the medulla of guinea pig was feather-like and in hamster, was egg-shaped. In hamster, it has a clear boundary in comparison to guinea pig. The medulla constitutes 51 % of large diameter and 22.5 % of small diameter in guinea pig and 43 % of large diameter and 40/14 % of small diameter in hamster. In guinea pig, reticularis cells penetrated into medulla and also the cortical invagination observed that formed around the blood vessels. So, structures also had been reported in bottlenose dolphin from the Adriatic Sea and it was explained that if organs grow permanently, the invagination can be from overgrowing of tissue during development (Vukovic et al. 2009). The cells with granules that are smaller and less electron-dense are epinephrine cells and those with larger and more electron-dense are norepinephrine cells (Steven and Lowe 1991).

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