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Histological, Histochemical, and Histoenzymatic Study of Pituitary Gland in Dog and Its Role in Animal Health

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ABSTRACT

Objectives: This study aims to examine the histological, histochemical, and histoenzymatic structures of the pituitary gland in dogs, which has limited research on the gland in dogs and its activity when compared with previous research.

Methodology: The samples obtained were subjected to a histological and histochemical study of the pituitary gland, which was collected from five dogs. The samples were fixed and processed, and microscopically evaluated by using H&E stain, and some of the specific stains for histochemical observation (H&E, PAS, Alcian blue, Van Gieson stains) and alkaline phosphatase for histoenzymatic analysis.

Results: The pituitary gland was found to have a thin connective tissue capsule made of collagen and a few elastic fibers surrounding it histologically. The adenohypophysis and neurohypophysis were the two regions it had. The adenohypophysis (anterior lobe) consisted of Pars distalis with mean intermediate diameter of thickness thickness (187.68 μ m) and housing the large numerous acidophils and smallest size basophils, and light-stained Homophones, Pars intermedia (intermediate lobe) which was the smallest region with smallest value of diameter (55.9 μ m), had a stratified layer of round oval basophilic cells and squamous cells. Some of these cells extended to the pars nervosa for a small distance. The pars nervosa (posterior lobe) represented the highest value of diameter (279.5200 \pm 38.96578 μ m) and was occupied by the neural tissue of the unmyelinated nerve fibers and various shapes of the pituicytes (glial cells) with acidophilic herring bodies. The collagen fibers stained red to brown in capsules, connective tissue with blood capillaries, and the elastic fibers stained black in colors with Van Gieson stains. The basophils differentiated from the acidophils by using the PAS and Alcian blue stains, which gave a positive reaction, while the acidophils gave a negative reaction. The cell membrane and cytoplasm of basophil cells in the Pars distalis showed exemplified alkaline phosphatase activity compared to the neurohypophysis.

Conclusions: The dog's pituitary gland was surrounded by a thin connective tissue capsule of collagen and elastic fibers, consisting of adenohypophysis and neurohypophysis, with the same subdivision as in the other animals, a prominent hypophysial cleft, and the pars intermedia was closely attached to the pars nervosa. Pars distlis contained large, numerous acidophil cells, smaller basophil cells, and paler chromophobes. Well-developed Pars intermedia was the smallest region with basophilic cells, squamous cells, and chromophobe cells. Pars nervosa was the largest region, consisting of a network of unmyelinated nerve fibers and numerous pituicytes. The basophils gave a positive reaction, and acidophils gave a negative reaction for PAS and Alcian blue. The Alkaline phosphatase enzyme activity in the pars nervosa gave positive reactions more than in other regions. These results reflect the healthy condition of the animals' status.

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Introduction

The dog was a domesticated descendant of the wolf. Also called the domestic dog, it was selectively bred from an extinct population of wolves during the late Pleistocene by hunter-gatherers. The dog was the first species to be domesticated by humans over 14,000 years ago, before the development of agriculture (John, 2022).

The pituitary gland has a major role in controlling important body functions; it is an endocrine gland; it has been identified as the master endocrine organ; its parenchymal cells of the pituitary gland synthesize certain hormones that regulate specific tissues or organs of the body (William, 1993; Greco, 2020). The system has many functions and presents slowly but persists for a long time (Vala *et al.*, 2013). These hormones that directly affect the activities of other endocrine glands in the animal body also have a significant purpose in the regulation of body metabolic rate, growth, and reproduction (Mahmood, 2014; Marieb & Hoehn, 2012). The pituitary lies in a minor shallow hypophysial fossa, "Sella turcica," a depression in the cavity of the basioccipital bone; it is covered with a thick fibrous sheet derived from dura mater through which the infundibulum stalk permits, communicating the gland with the hypothalamus (Trautman, 1909b). The pituitary gland comprises three important parts (anterior, posterior, and intermediate lobes). The anterior lobe (pars buccalis) was the major and largest lobe of the equine hypophysis; the anterior lobe of cattle, humans, rats, and rabbits was greater than the posterior lobe (Herring, 1908). Dura mater covers the Major parts of the gland except for an opening through which the hypophyseal stalk passes (Dyce *et al.*, 1996).

The pituitary gland (hypophysis) in mammals is considered an appendage of the brain, which clarifies the relationship between the nervous and humoral mechanisms that control certain functions (Bayram-Weston *et al.*, 2021).

In all vertebrates, the pituitary gland is composed of two parts: the glandular portion (adenohypophysis) and the neural portion (neurohypophysis), which are joined and encapsulated together. The adenohypophysis is divided into pars distalis, pars tuberales, and pars intermedia. The neurohypophysis is divided into the pars nervosa and the infundibulum (Ilahi & Ilahi, 2021). The pars distalis and pars tuberalis are the anterior lobe of the gland, while the pars nervosa and pars intermedia are termed the posterior lobe (Liebich, 2019).

The different cell types of the pituitary gland are identified by the characteristic arrangement, distribution, and staining properties of different cell types (Holmes & Ball, 1974). Because of the little information about the pituitary gland in the local breed of dog, our research focused on the histological, histochemical, and enzymatic structures of some parts and cells.

Materials and Methods

Ethical approval

Compliance with ethical guidelines

Ethical approval was obtained and accepted from the scientific research ethics committee of the College of Veterinary Medicine, University of Basrah, Basrah, Iraq, numbered 114/37/2026 on 5/1/2026.

Funding

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Authors' contributions

All authors contributed equally to the conception and design of the study, data collection and analysis, interpretation of the results, and drafting of the manuscript. Each author approved the final version of the manuscript for submission.

Conflict of interest

The authors declared no conflict of interest.

Samples collection

The dog pituitary glands used in this study were dissected from five dogs that were used in the anatomy and histology department in the College of Veterinary Medicine at Basrah University. The obtained samples were subjected to histological, histochemical, and histoenzymatic studies. The pituitary glands of dogs were dissected by using a saw and liberation of it from the ventral side of the head region. The mandible was first removed after the styloid process of hyoid bone was broken, the basisphenoid bone was gently cut and removed, then using scalpel to cut the dura lining after that the gland removed from its position in the sella turcica (Nasr *et al.*, 2021).

Tissue preparation

The collected specimens were reserved in 10% formalin for 2-3 days, then dehydrated in serial concentration of ethanol alcohol begun from the 70%- 100% absolute alcohol, the cleared with two changes of xylene, after that infiltrated and embedded in paraffin wax and trimming with 5-6 microns thickness and mounting on the glass slide and stained with Harris's haematoxylin and Eosin (Suvarna *et al.*, 2018) for the general histological structures. Periodic acid Schiff stain, Alcian blue for detection of acidic mucopolysaccharides (Pearse, 1968). Van Gieson stain was used to demonstrate the

collagen fibers, and the measurement of alkaline phosphatase activity was used in this study. that accumulated (Fig.4).

Statistical analysis

To study the differences in the diameter of lobes of the pituitary gland in five dogs and compare with the previous study in normal status, the mean and standard deviation were used to display the results. Using the statistical software program (SPSS for Windows version 22, USA), the data were statistically analyzed using One-Way ANOVA (Tukey HSD) with multiple comparison tests. ($P < 0.05$) difference was considered significant.

Results

Histological findings

In dogs, this gland consists of two prominent lobes: the outer anterior lobe (Adenohypophysis) and inner posterior lobe (Neurohypophysis). And the third one was the smaller lobe intermediate lobe located between them, The statistical analysis of these lobes revealed significant differences at level ($P < 0.05$) in their diameter, the anterior lobe consisted of two regions, Pars distal and Pars tuberalis with diameter ($224.6600 \pm 42.75714 \mu\text{m}$), while the intermediate lobe involved the Pars intermedia was having the smallest value of diameter ($55.9000 \pm 10.96289 \mu\text{m}$) in compared to the diameter of pars nervosa (posterior lobe) was the largest value (279.5200 ± 38.96578) (Table 1) and (Figure 1), Pars distalis was separated from the Pars intermedia by a prominent hypophysial cleft, while the Pars intermedia and Pars nervosa were attached together without remarkable lines. The Pars distal, Pars intermedia, and Pars nervosa were explained in this study (Fig. 2). A thin connective tissue capsule composed of collagen fibers and a few elastic fibers was the first layer that enveloped the pituitary gland (hypophysis) (Fig. 3).

Pars distalis

The parenchyma of the Pars distalis appeared in the form of clusters or cords of cells; some of these joined together. These cells were divided according to their affinity to the dyes into two groups: the chromophils and the chromophobes. The chromophils consisted of two types according to the granules that were observed within the cytoplasm of the cells: the large ovoid to polygonal-shaped and pink cells with heterochromatic nuclei, and an acidophilic cytoplasm were called acidophils, while the other cells were smaller than the acidophils and had a round-to-oval shape with dark center nuclei. The chromophobe's cell was stained weakly and appeared pale in color, with dark nuclei of irregular shape

Pars tuberalis

The pars tuberalis was a thinner portion surrounding the infundibular stalk and extended from the pars distalis. The parenchymatous cells are organized as a cluster (nest) and follicles surrounded by blood capillaries and contain large secretory granules. Some of the basophils of thyrotrophic and gonadotrophic cells of the Pars distalis have been seen in this portion with squamous cells, magenta syncytial cells, and clear cells (Fig. 5).

Pars intermedia

Pars intermedia (intermediate lobe) was located close to the Pars nervosa as a narrow zone of cells with a mean, and represented the smallest value among the other parts. It was well developed, and the cells were arranged as cords of stratified layers of cells and seemed to invade some distance from the Pars nervosa. Some of these cells were oval to round in shape, with dark basophilic cytoplasm, called magenta cells, flattened squamous cells, and a few chromophobes with light or pale staining, which were devoid of cytoplasmic granules in H&E stain (Fig.6).

Pars nervosa

The neural tissue was the main composition of the Pars nervosa (posterior lobe) and consisted of the unmyelinated nerve fibers that scattered and radiated in various directions, different shapes of pituicytes (glial cells) with different shapes of nuclei (oval, elongated), and sizes that were scattered and distributed among the nerve fibers and between the blood capillaries that were numerous with the Pars nervosa. In addition, the secretory granules appeared as accumulated homogenous and eosinophilic staining bodies called Herring bodies with H&E stain (Fig. 7).

Histochemical observations

The connective tissue capsule that surrounded the pituitary gland contained a connective tissue fiber, predominantly collagen fibers, which were colored red to brown, and a few elastic fibers that stained black. The collagen fibers were also located surrounding the sinusoidal in the pituitary gland regions, particularly in the Pars nervosa with van Gieson stain (Fig. 8). The Alcian blue stain used recognized the basophil cells (gonadotrophin that secretes the F.S.H, L.H, and thyrotroph that secretes T.S.H.) rather than acidophilus cells (somatotroph and lactotroph) in the anterior lobe of the pituitary gland, in which the glycoprotein in secretory granules of basophil gives a positive reaction to the stain and appears bluish green to turquoise color. In contrast,

the acidophilus cells give negative reactions and are not stained, but the chromophore gives a light color and can also demonstrate the connective tissue with the regions of the gland around the blood capillaries (Fig.9). The basophil cells stained magenta and gave a positive reaction to the PAS reaction, especially thyrotrophs, and can differentiate from acidophil cells that gave a negative reaction, and the chromophores are visible as paler-stained cells (Fig. 10).

Histoenzymatic findings

The Alkaline phosphatase enzyme activity was higher in the posterior lobe (Pars nervosa), which concentrated in the capillary wall and organelles of the pituicytes, and the moderate density was located in the Pars distalis, especially in the gonadotroph cells, but the lower density was in the Pars intermedia (Fig. 11, 12, 13, 14).

Table 1: The mean diameter of lobes in the pituitary gland of dogs:

Mean of the pituitary gland in dogs	Mean ±SD / μm Anterior lobe	Mean ±SD/ μm Intermediate lobe	Mean ±SD/ μm Posterior lobe
	224.6600±	55.9000±	279.5200±
	42.75714 ^B	10.96289 ^C	38.96578 ^A

* A, B, and C significant differences in the lobes of the pituitary gland in the dog.

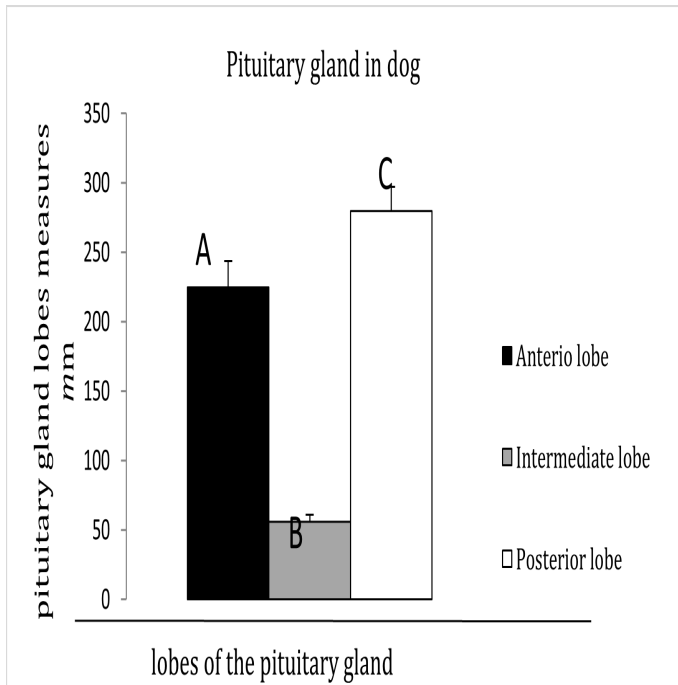


Figure 1: Chart show the significant differences in diameter measures of lobes of pituitary gland in doge (A) Anterior lobe with intermediate value (B) Intermediate Lobe with lower value (C) posterior lobe with higher value.

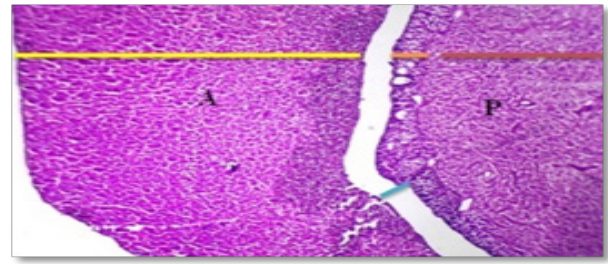


Figure 2: Histological structures of the pituitary gland in the dog show the its parts, A: Anterior lobe, P: Posterior lobe, Pars distalis (yellow line), Pars Intermedia (orange line), Pars nervosa (Red line), Hypophyseal cleft (blue line). H&E stain 4x.

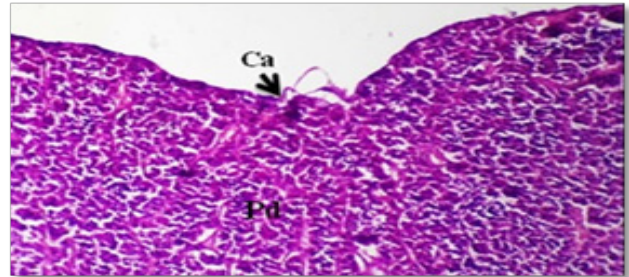


Figure 3: Histological structures of the pituitary gland in dog show the fine connective tissue capsule that surrounds the gland (Ca), in the Pars distalis (Pd).H&E. stain. 10X.

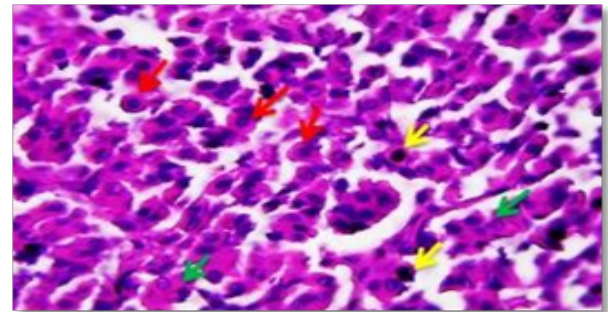


Figure 4: Histological structures of the pars distalis in the anterior pituitary gland of the dog show: Acidophil cells (Red arrow), Basophil cells (Yellow arrow), Chromophobes (Green arrow). H&E. Stain.

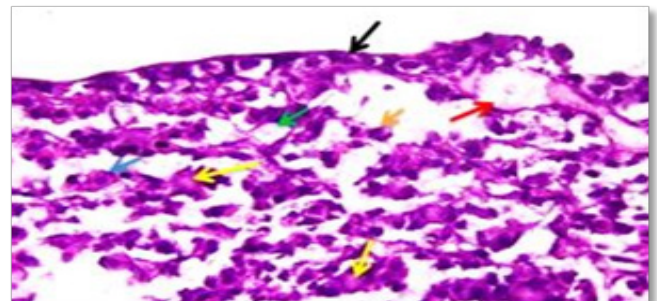


Figure 5: Histological structures of Pars tuberalis in the pituitary gland of the dog show: Squamous cells (black arrow), Basophil cells (blue arrow), clear cells (Green arrow), Magenta syncytial cell (orange arrows), Blood sinusoid (red arrow), secretory bodies (yellow arrows). H&E. Stain. 40X.

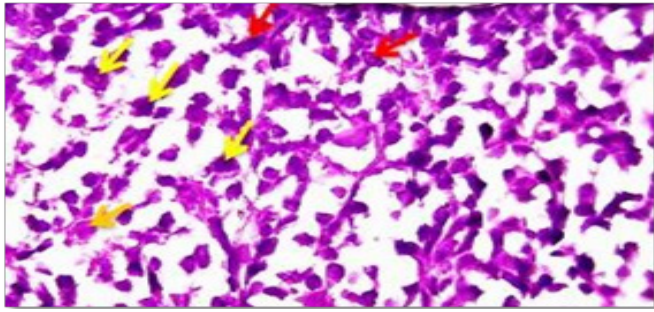


Figure 6: Histological structures of Pars intermedia of intermediate lobe of pituitary gland in dog show; Magenta cells (yellow arrow), Squamous cells (red arrow), chromophobes (orange arrow). H&E. stain 40X

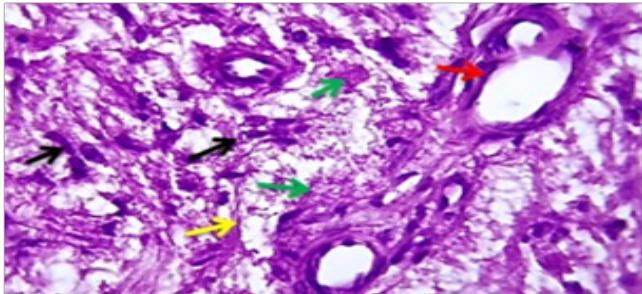


Figure 7: Histological structures of Pars nervosa in posterior lobe of pituitary gland in dog show: Pituicytes (Black arrow), Unmyelinated fiber (Yellow arrow), Herring body (Green arrow), Blood capillaries (Red arrow). H&E stain 40X.

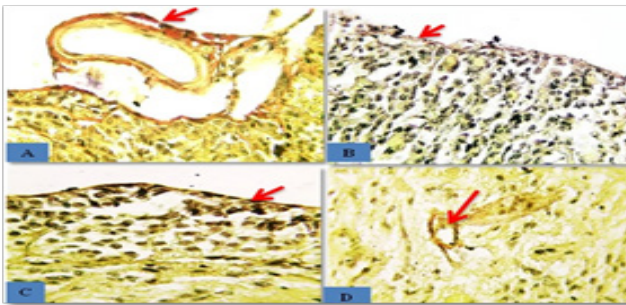


Figure 8: Histochemical section shows the collagen fibers (Red arrow), and few amounts of elastic fiber (Black arrow) in (A), (B) pars distalis, (C) Pars intermedia, (D) pars nervosa. Van Gieson stain. 40X.

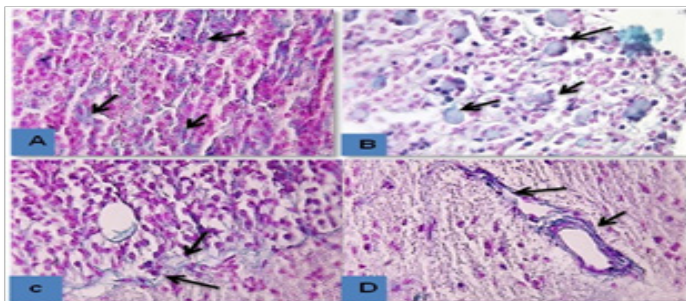


Figure 9: Histochemical section of pituitary gland of dog show: (A) Pars distalis with basophil cells, (b) Pars intermedia with connective tissue at the junction with Pars nervosa, (C) Pars nervosa with connective tissue in the blood capillaries, Alcian blue stain. 40X.

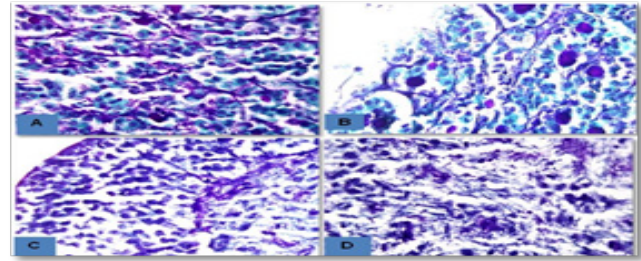


Figure 10: Histochemical section anterior lobe of pituitary gland of dog show: Pars distalis with positive staining basophil cells that staining magenta in color (Red arrow) and negative acidophil cells (Yellow arrow), (B) Pars tuberalis with secretory bodies (red arrows) and basophil cells (black arrow), (C) Pars intermedia, (D) Pars nervosa with herring bodies (Black arrow). PAS stain 40X.

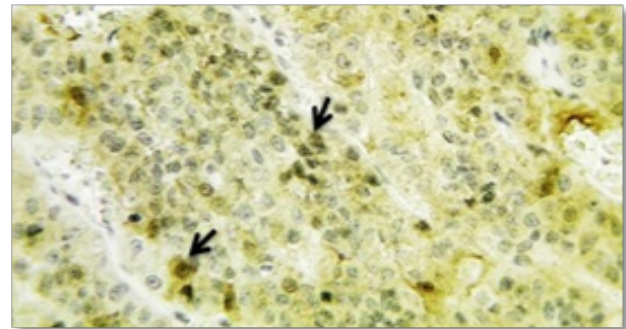


Figure 11: Show the moderate activity of the Alkaline phosphatase enzyme in gonadotrophes cells in Pars distalis of anterior lobe of pituitary gland in dog. Alkaline phosphatase. 40X.

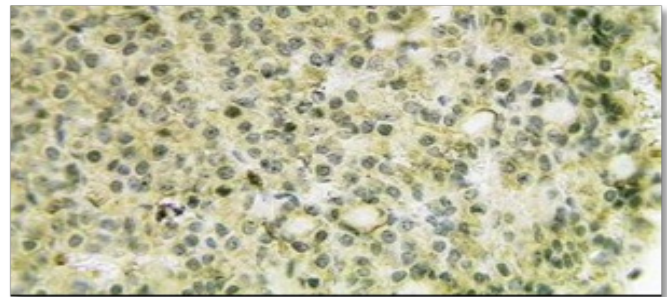


Figure 12: Show the weak activity of the Alkaline phosphatase enzyme in cells in Pars tuberalis of anterior lobe of pituitary gland in dog. Alkaline phosphatase. 40X.

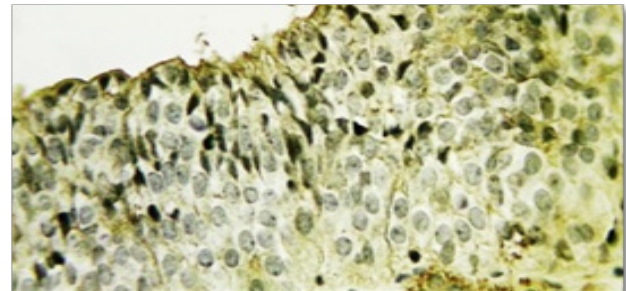


Figure 13: Show the weak activity of the Alkaline phosphatase enzyme in gonadotrophes cells in Pars intermedia of posterior lobe of pituitary gland in dog. Alkaline phosphatase. 40X.

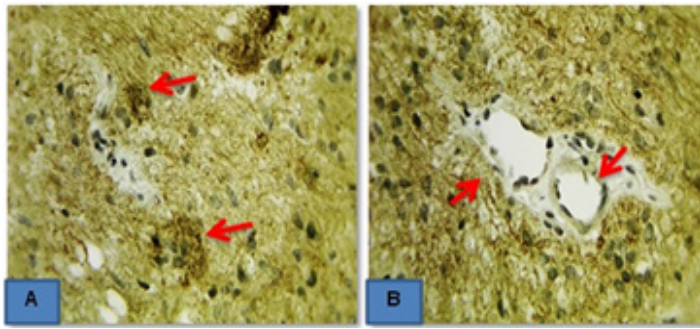


Figure 14: Shows the strong activity of the Alkaline phosphatase enzyme in Pars nervosa of the posterior lobe of the pituitary gland in a dog (A) Herring bodies (Red arrows), (B) around the blood vessels (Red arrows). Alkaline phosphatase. 40X.

Discussion

In this study, the histological observation found that the pituitary gland in dogs consisted of the anterior adenohypophysis and the posterior neurohypophysis. The adenohypophysis was divided into Pars distalis and Pars tuberalis, and the neurohypophysis involved Pars intermedia and Pars nervosa. These findings were as described in other species of animals in (Banks, 1993; Bacha & Bacha, 2000; Vuković *et al.*, 2011) in dolphins and (Manojlović-Stojanoski *et al.*, 2007; Alnoor *et al.*, 2024) in rabbits.

Our result clarified that the capsule that covered the pituitary gland consisted of thin connective tissue, containing mainly collagen fibers and a few elastic fibers. This agrees with (Nasr *et al.*, 2021) in donkeys. The pars distalis have intermediate thickness and this finding disagreement with (Mahmood, 2014), it consisted of the acidophil cells appeared large rounded to oval in shape, numerous in number with eosinophilic cytoplasm similar to (Gartner & Hiatt, 1997) in human and dissimilar to (Chandana *et al.*, 2025) in sheep that mentioned the acidophil have pyramid shape and accumulated to gather to form tight clump, The basophil cells that smaller than acidophilic and have different shape with basophilic cytoplasm this agreement with (Banks, 1993) in domestic mammals, the chromophobes that were fewer in number and have irregular shape this result was align with other domestic animals (Jaspal *et al.*, 2019) in camel, Nasr *et al.*, 2021) in donkey, the our finding disagree with (Mahmood, 2014) that reported the largest cells was the chromophobes and acidophils were smallest in pars distal of rat. Pars tuberalis consisted of squamous cells as a nest, basophil cells that migrated from the pars distalis, with clear large cells, magenta syncytial cells with secretory granules. This finding corresponds to (Gartne & Hiatt, 2006). Pars intermedia was well developed, as in (Al-Mansour & Hussein, 2025), in New Zealand Rabbits. It consisted of numerous cells of magenta, basophil, and chromophobe cells, which were arranged in multilayers of a stratified type. The mean thickness of this region was (55.9 μm), this result corresponding to the

findings with (Banks, 1993) and (Trautmann & Fiebiger, 2015) in domestic mammals and differs from the report of (Oishi *et al.*, 1992) that the mouse pars intermedia was larger compared to other species. Pars nervosa contained a network of unmyelinated nerve fibers with pituicytes that were located around the blood capillaries and surrounded by connective tissue with the presence of herring bodies that contained basophilic secretory granules. These results were consistent with those (Mahmood, 2014) in rats, (Beheiry & Goda, 2022) in camels, and (Pathak & Bansal, 2017) in Indian buffalo. In this study, the pars nervosa showed the largest lobe, which disagrees with Mahmood (2014) in rats. Other previous research recorded that the pars nervosa showed hypocellularity in humans and mice (Kelberman *et al.*, 2009). The Alkaline phosphatase enzyme activity gave a positive reaction with different degrees of staining density, and higher density was observed in the pars distalis because it has different cells that produce different hormones. This finding agrees with (Yamaguchi *et al.*, 1981) in various mammals and (Yamaguchi, 1994).

Conclusion

The pituitary gland in the dog has an important role in the animal's health status. Studying this gland indicates the activity of cells of this gland that produce and secrete many hormones affecting the body's health. Histologically surrounded by thin connective tissue capsules of collagen and elastic fibers that gave a positive stain to the Van Gieson stain. It was divided into the adenohypophysis and neurohypophysis; the adenohypophysis consisted of the Pars distalis and Pars intermedia with mean thicknesses of 187.68 μm and 55.9 μm , respectively. Neurohypophysis involved the largest region in the gland: Pars nervosa, with a mean thickness of 279.52 μm . Pars distlis contained numerous acidophil cells, smaller basophil cells, and paler chromophobes, well-developed Pars intermedia with basophilic cells, squamous cells, and chromophobe cells. Pars nervosa consisted of a network of unmyelinated nerve fibers, numerous pituicytes, supporting connective tissue, and numerous herring bodies with acidophilic secretory granules. The basophils were recognized by acidophils by using PAS and Alcian blue stains, in which basophil cells gave a positive reaction, and acidophils gave a negative reaction. The Alkaline phosphatase enzyme activity in the pars nervosa gave positive reactions around the capillary wall and pituitary cell membrane and organelles, more than in other regions.

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