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Histological and immunohistochemical studies of the adrenal cortex in she-camel with special reference to age variation

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ABSTRACT

The adrenal glands, responsible for regulating important body functions. This study aimed to examine the structure of the adrenal cortex in 15 healthy she-camels. The camels were divided into three groups based on their age: immature 1-2, mature 3-7, and senile up to 12 years which are not equally divided. Camel adrenal glands are enveloped by a dense network of collagen fibers, forming a unique capsule. This capsule comprised an outer fibrous layer and an inner cellular layer. Notably, connective tissue trabeculae originating from the inner side of the capsule invaded the gland's cortical parenchyma, giving the glomerulosa a distinctive arrangement. This arching system was prominent in mature and senile camels but absent in the immature ones, where eosinophilic cells formed clusters resembling follicles. Hematoxylin and Eosin (H&E) staining was used for general tissue visualization, Masson's trichrome (MTC) staining for collagen fiber, and orcein staining for elastic fiber visualization. Immunohistochemical analysis using caspase3 as an apoptotic marker and proliferating cell nuclear antigen (PCNA) as a marker for cell proliferation shed light on cellular behavior. The immunohistochemical staining revealed strong positive nuclear reactions of PCNA in immature and senile camels, respectively, and weak responses in mature camels. Additionally, caspase 3 exhibited cytoplasmic and nuclear reactivity, transitioning from weak positive in mature camels to strong positive in both immature and senile stages. In conclusion, our study unraveled interesting similarities and distinctive features of camel adrenal glands, providing insights into their architecture.

1. INTRODUCTION

The camel, a fascinating creature, belongs to the Camelidae family, which encompasses the sub-family Cameline, which consists of two distinct genera. The genus Camelus comprises two extraordinary species: Camelus dromedarius, known as the dromedary, one-humped or Arabian camel, and Camelus Bactrians, recognized as the Bactrian or two-humped camel. The second genus contains the llama, a captivating creature with four species: the domesticated llama, llama pacos, and alpaca; the wild llama guanaco, and the llama vicugna, each exhibiting unique characteristics (Khanna et al., 2004). Recognized as the "desert ship," camels have evolved remarkable adaptations that enable survival in harsh desert environments. These adaptations are intricately linked to the activity of specific endocrine glands, including the adrenal glands, which play a pivotal role in numerous vital functions (Ibrahim et al., 2017) .

Camels have long served as invaluable multipurpose animals, contributing to various aspects of human life. They have been utilized for transportation, providing reliable means of traversing vast distances. Additionally, camels have been prized for their meat and milk production, offering sustenance to communities in arid regions. Furthermore, their wool and hide have served as valuable byproducts, supporting the creation of essential textiles and durable materials (Emam et al., 2013 and Zaky et al., 2020) .

Adrenal glands, complex endocrine organs, govern many physiological processes crucial for overall health. These include stress response, metabolism, immune functions, and cardiovascular dynamics. Disruption in normal adrenal gland function can lead to severe metabolic imbalances, circulatory collapse, hypoglycemic coma, and even mortality across various mammalian species (Igbokwe and Jacobs, 2016). Cortisol, a hormone produced by the adrenal cortex, is a crucial indicator of stress. Increased plasma cortisol levels have been observed following transportation and hypoxemia in new-world camelids, while cortisol levels remain unaffected by food deprivation in dromedary camels (Dahl born et al., 1992; Riquelme et al., 1998 and Anderson et al., 1999). The adrenal glands are pivotal in maintaining homeostasis, regulating fetal organ maturation, and initiating parturition in females. Furthermore, the unique biological structure of camel adrenal glands is believed to be instrumental in their remarkable adaptation to extreme environments (Ye et al., 2017). Camels possess bilateral adrenal glands in the retroperitoneum, superior and above the kidney. The left adrenal gland typically exhibits a flat and oval shape, while the right adrenal gland assumes a triangular form. Notably, the right adrenal gland may have the liver near or partially covering its inner side, resulting in distinct liver markings (Ye et al., 2015). Adrenal gland histology has been extensively studied in human and diverse animals, including cattle, buffalo, goat, pig, camel, and

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geese. These investigations have contributed valuable insights into glandular structure and function. Among these Species are; cattle (Jelinek and Konecny, 2011 and Igbokwe and Jacobs, 2016), buffalo (Sarwar Qureshi, 2007; Fouad Attia, 2013 and Kumar et al., 2021), goat (Hussein, 2019 and Suri et al., 2022), pig (Suzuki and Kachhi, 1994), human(Kimura et al., 2005 and Kampan and Fluck, 2008), geese (Elzoghby, 2015), camel (Al-Bagdadi, 1969; Khanna et al., 2004; Rehan and Qureshi, 2007 and Ye et al., 2017) .

Each adrenal gland exhibits two distinguishable structures: the outer adrenal cortex and the inner medulla. The cortex comprises three distinct zones with unique histologic features and hormone synthesis capabilities. The zona glomerulosa (ZG) contains small angular cells dispersed under the capsule, producing the primary mineralocorticoid, aldosterone. The major component, the zona fasciculata (ZF), consists of large clear lipid-laden cells arranged in columns from the capsule to the inner zona reticularis (ZR). Recent studies suggest ZF is the primary source of glucocorticoids, such as cortisol, in human glands. The ZR, composed of eosinophilic compact cells with minimal lipid storage, forms cord-like structures around vascular sinusoids. This zone can also produce cortisol and appears to be the source of androgens (McNicol, 2008 and Kampan and Fluck, 2008). Moreover, Immunohistochemistry studies commonly employ specific antibodies to investigate cellular processes. Antibodies against caspase 3 and PCNA have been proven valuable in immunohistochemical analyses. Caspases, crucial components of apoptosis, are important indicators of programmed cell death, while PCNA is a marker for cell proliferation and an indicator of adrenal disorders (Van Diest et al., 1998) .

Overall, the present study aimed to investigate the histological structure and immunohistochemical changes of the camel adrenal cortex in different age groups.

2. MATERIAL AND METHODS

2.1. Animal Selection :

The study involved the examination of adrenal glands from 15 she-camels Camelus dromedaries. The camels were divided into three age groups: aged (more than 12 years), mature (3-7 years), and immature (1-2 years). The number of specimens in each age group was determined based on previous research by Al-Bagdadi, (1969). There were 5 specimens in the aged group, 6 in the mature group, and 4 in the immature group .

2.2. Sample Collection :

The adrenal glands were collected from each she-camel after veterinary-hygienic inspection to prevent abnormal diseases. The collection was performed by opening the abdominal region and carefully separating the adrenal glands just above the kidneys. The camels were obtained from a slaughterhouse in Toukh, Egypt, and the collected samples were brought to the Department of Histology, Faculty of Veterinary Medicine, Benha University, for analysis .

2.3. Histological Examination:

The collected adrenal gland specimens were fixed in 10% neutral buffered formalin for 48-72 hours (about 3 days). Following fixation, the specimens underwent a series of dehydration steps using increasing concentrations of alcohol. Subsequently, the specimens were cleared in xylene and embedded in paraffin wax. Thin sections of 5µm thickness were prepared using a microtome (Suvarna and Bancroft, 2019)

Various staining techniques were employed to visualize the histological structure and specific components of the adrenal glands. Harris's Hematoxylin and Eosin stain was commonly used for general histological examination and assessment of tissue morphology. Masson's trichrome stain was used to highlight collagen fibers, while Orcein stain was utilized to identify elastic fibers.

2.4. Immunohistochemistry :

Paraffin sections with a thickness of 5 micrometers were collected from the adrenal gland and mounted on positively charged microscope slides. The sections were deparaffinized by incubating them in xylene, followed by sequential rehydration in absolute ethanol, 95% ethanol, 70% ethanol, and distilled water. The tissue sections were heated in a 10 mM citrate buffer (pH 6.0) for 40 minutes for antigen retrieval. Subsequently, the sections were allowed to cool to room temperature for 20 minutes. To reduce peroxidase activity, the sections were treated with 3% hydrogen peroxide in methanol for 15 minutes. To block non-specific staining, the sections were incubated with 10% normal goat serum for 1 hour at room temperature. The sections were incubated overnight at 4°C with rabbit polyclonal caspase 3 antibodies (catalog number C9598, Sigma Aldrich, St. Louis, Missouri, USA) diluted at 1:1000 and mouse monoclonal PCNA antibodies (catalog number P8825, Sigma Aldrich, St. Louis, Missouri, USA) diluted at 1:500. Following the primary antibody incubation, the sections were treated with a human anti-rabbit and mouse secondary antibody for caspase-3 using indirect immune peroxidase technique and PCNA for 1-2 hours at room temperature. For PCNA antibody staining, the sections were incubated overnight at 4°C with. The immunohistochemical staining was visualized according to the method outlined by Boenisch (2009)

2.5. Histomorphometry :

Image analysis was performed using "Image J" software (version 1.48v, National Institute of Health, USA). For quantitative evaluation, ten different non-overlapping randomly selected fields were examined from each slide and the following parameters were assessed :

The mean thickness of the adrenal cortex (µm) and its capsule was determined from Hematoxylin and Eosin (H&E)-stained sections at 10X .

The mean area percentage of collagen fiber content was analyzed from Masson's trichrome-stained sections at 10X .

The mean expression of caspase-3 and PCNA immunohistochemical positive cells is calculated as the average of a mean grey value. This analysis was conducted on 3,3'-Diaminobenzidine (DAB)-stained sections at 10X -.

These quantitative parameters provided valuable insights into the adrenal gland's structural characteristics and cellular dynamics .

2.6. Statistical analysis :

The data are presented as means ± SD. Statistical analysis was conducted using PRISM GraphPad version 9.0 (GraphPad Software, San Diego). One-way analysis of variance (ANOVA) was employed to compare multiple data sets. In cases where the obtained P value from ANOVA was significant, Tukey's test was utilized to determine the

differences among the groups. A significance level of $P < 0.05$ was considered statistically significant.

3. RESULTS

3.1. Histological observations

The adrenal gland is encircled by a connective tissue capsule characterized by two layers: an outer fibrous layer and an inner cellular layer. There are some fat cells present in the cellular layer of the capsule in immature camels that decrease in number toward senile age (Figure 1). The outer layer of the capsule is composed of dense connective tissue rich in collagen and elastic fibers (Figure 2). Immature age groups exhibit higher collagen fibers than mature and senile age groups, as revealed by Masson's trichrome staining (Figure 2A, 2B, 2C). Thick trabeculae extend from the inner side of the capsule into the cortex to varying degrees (Figure 2D, 2E, 2F). The proportion of fibrous tissue area is higher in senile camels compared to immature and mature ones (Table 1). When stained with orcein, the elastic fibers within the capsule are more prominent in the immature age group compared to both mature and senile age groups (Figure 2G, 2H, 2I). The zona glomerulosa (ZG) arrangement in Camels is unique because of the trabeculae; they create an arching structure, with the limbs of the arches separating the glomerulosa units into groups that vary greatly in size and quantity. The eosinophilic cells are arranged in tiny masses or balls that resemble follicles in the immature camels' zona glomerulosa, which lacks an arching system (fig.3A). However, in mature and senile camels, the arching system becomes visible (Figure 3B, 3C). The cytoplasm of the cells is vacuolated in immature camels, granulated in mature age, and less granulated in senile age. The zona fasciculata (ZF) is the largest zone within the cortex of the adrenal gland, including polyhedral cells. Most of them are called spongiocytes, which have granular vacuolated cytoplasm and a spherical centrally located nucleus (Figure 3D-F). They are organized in cords of one or two cells, with some forming vertical columns and others lacking a defined orientation. These cellular cords are separated by vascular sinusoids. Notably, the cells of the zona fasciculata display increased vacuolation in immature adrenal glands compared to mature and senile glands (Figure 3D). Conversely, mature and senile adrenal glands exhibit more granulated cytoplasm (Figure 3E, 3F). Moreover, in senile adrenal glands, numerous and larger blood sinusoids exist between the cells, unlike the immature and mature stages (Figure 3F). The zona reticularis (ZR) occupies the innermost portion of the cortex. The cells within the zona reticularis are cuboidal to polyhedral in shape and form a net-like arrangement of anastomosing cords, typically one to two thick cells. In the immature adrenal glands, these cells are arranged around wider blood sinusoids (Figure 4A).

Like the zona fasciculata, the cells of the zona reticularis exhibit increased vacuolation in immature glands (Figure 4A) compared to mature and senile glands. However, in mature adrenal glands, the cells display a granulated and eosinophilic appearance (Figure 4B), while in senile glands, the granulation is less pronounced (Figure 4C).

3.2. Immunohistochemical findings

3.2.1. PCNA:

Immunohistochemical analysis using PCNA revealed distinct reactivity patterns in camels' adrenal cortex at different ages. In all ages, certain adrenal cortex cells exhibit positive reactivity, while others exhibit negative reactions. In immature camels, most cells exhibited strong nuclear reactions in (ZG) (Figure 5A), (ZF) (Figure 5D), and (ZR) (Figure 5G). Also, senile camel's adrenal cortex shows strong positive nuclear reaction in ZG (Figure 5C), ZF (Figure 5F), and ZR (Figure 5I). In contrast, the adrenal cortex of mature camels showed a weak positive reaction to PCNA, ZG (Figure 5B), ZF (Figure 5E), and ZR (Figure 5H). Immunohistochemical quantification demonstrated a significant decrease in PCNA expression in mature animals compared to immature camels (Table 1).

3.2.2. Caspase-3:

In the adrenal cortex, the immunohistochemical staining of caspase 3 protein revealed distinctive cytoplasmic and nuclear reactions in different age groups. In immature camels, there is strong positive reaction to caspase 3 in ZG (Figure 6A), ZF (Figure 6D), and ZR (Figure 6G). Also, the senile camels show strong positive reaction, like what happens to immature ones in ZG (Figure 6C), ZF (Figure 6F), and ZR (Figure 6I). On the other hand, the adrenal glands of mature camels displayed a weak positive reaction to caspase 3 in ZG (Figure 6B), ZF (Figure 6E), and ZR (Figure 6H). Immunohistochemical quantification revealed a significant decrease in caspase-3 expression in mature animals compared to immature and senile animals (Table 1).

Table 1: Histological parameters of the adrenal gland in she-camels at different age stages.

Parameter	Immature	Mature	Senile
	Mean ± SD		
Capsule thickness (µm)	35.9 ± 6.3	31.3 ± 6.9	22.2 ± 3.6 ***
Fibrous tissue area (%)	17.2 ± 4.3	19.6 ± 5.7	25.9 ± 7.9 **
PCNA expression	179.8 ± 0.5	178.4 ± 1.1*	177.7 ± 0.8
Caspase-3 expression	171.5 ± 2.8	159.1 ± 5.1 **	172.6 ± 2.3

*= p<0.05 (significant)
** = p<0.01 (very significant)
*** = p<0.001 (extremely significant)
**** = p<0.0001 (extremely significant)
In some cases, asterisks may also be used to indicate:
= p<0.1 (trend towards significance)
† = p<0.15 (weak trend)

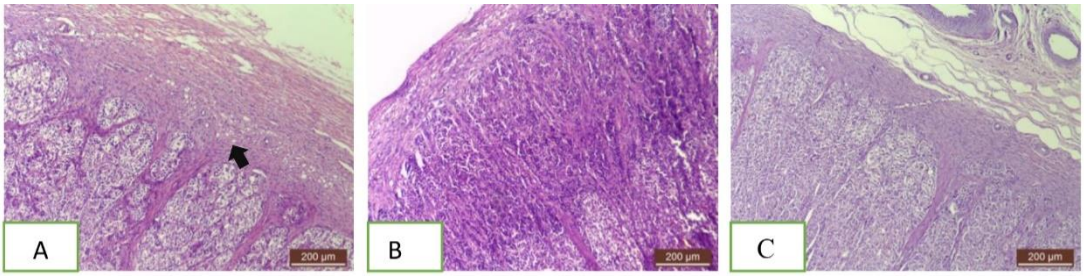

Figure 1: Photomicrograph showing the thick capsule and presence of some fat cell (arrow) in the adrenal gland of immature camel (A), mature camel (B), and senile camel (C). H&E.

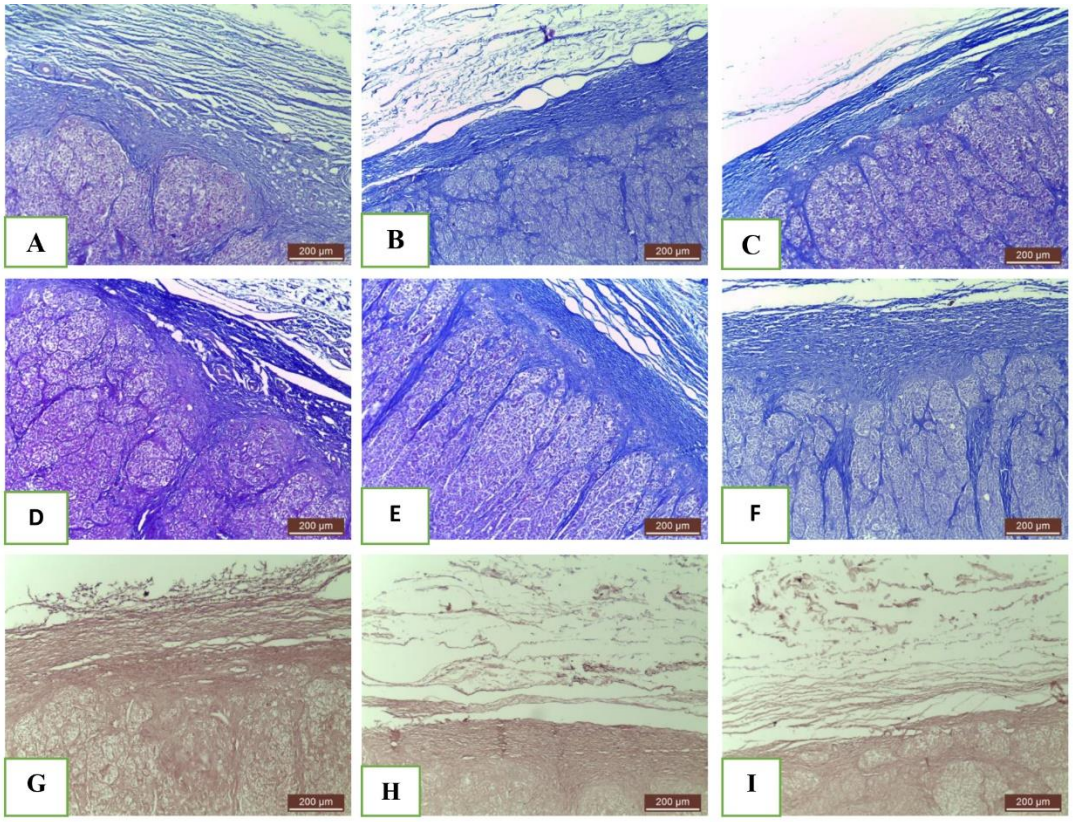

Figure 2: Photomicrograph showing the outer fibrous layer which consists of collagen and elastic fiber. (A) High level of collagen fibers of the capsule in immature age compared to mature (B) and senile (C). Masson's trichrome stain (MTC). (D),(E),and (F) Thick trabeculae enter from the inner side of the capsule into the substance of the cortex to different extents. MTC. (G) High level of elastic fibers of the capsule in immature age compared to mature (H) and senile (I). Orcein.

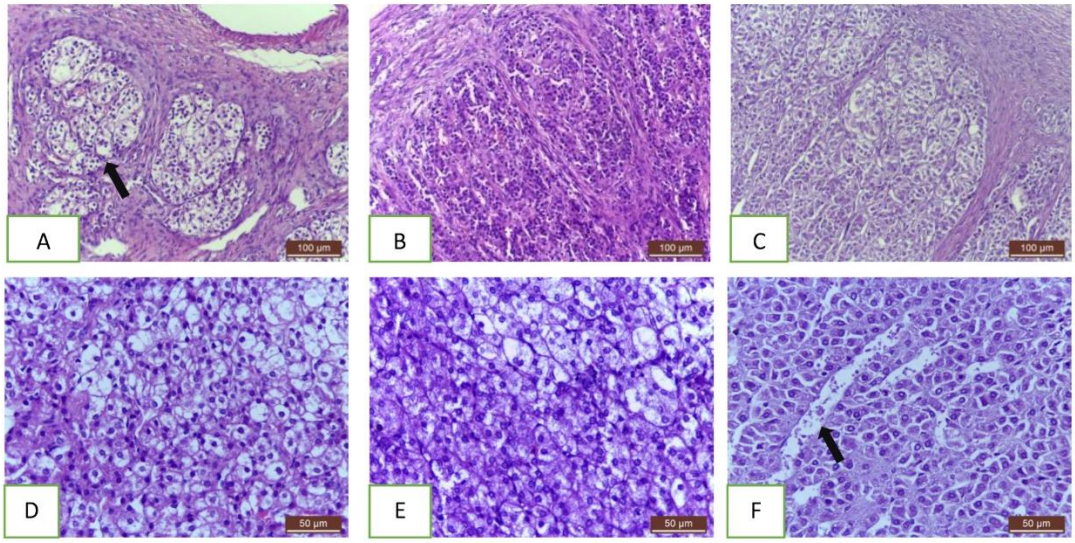

Figure 3: Photomicrograph showing zona glomerulosa (ZG) and zona fasiculata (ZF). (A) ZG of immature camels arranged in small masses or balls (arrow). Arching system of ZG of mature (B) and senile camels (C). (D) Cells of ZF in immature which are more vacuolated compared to mature (E) and senile ones (F), both of them have granulated cells, and there is numerous and wider blood sinusoid (arrow) between cells in senile camels. H&E.

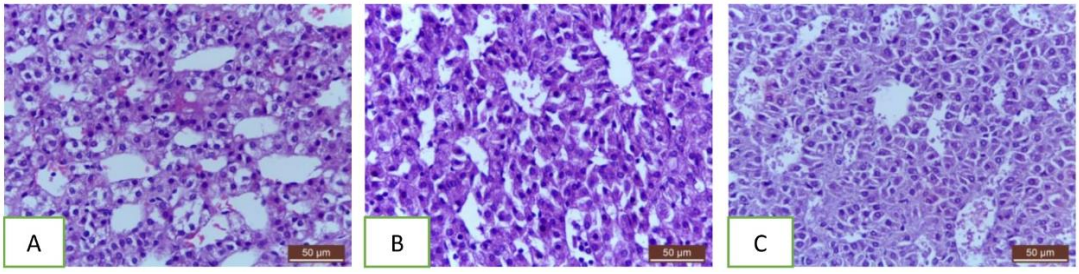

Figure 4: Photomicrograph showing zona reticularis (ZR). Vacuolated cells of ZR arranged around wider blood sinusoid in immature camels while in mature (B) and senile camels (C) the cells are granulated. H&E.

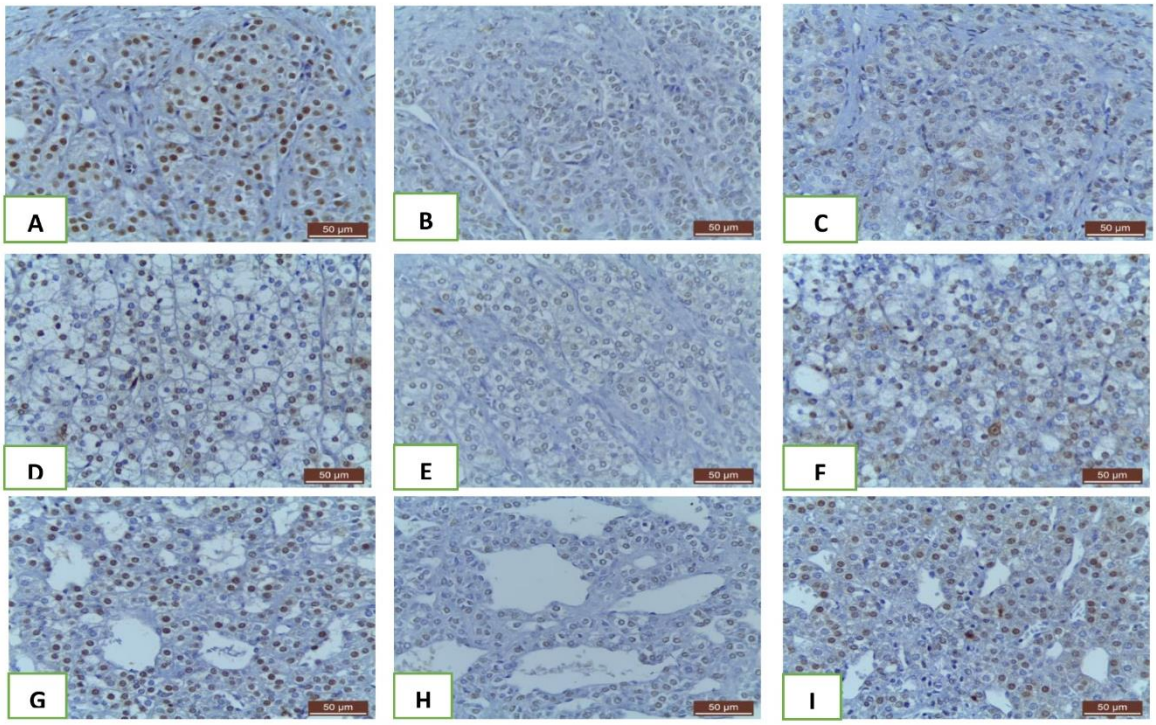

Figure 5: Photomicrograph showing immunohistochemical staining in the adrenal cortex using PCNA. Strong positive nuclear reaction in ZG (A), ZF (D), and ZR (G) of immature, as well as in ZG (C), ZF (F), and ZR (I) of senile camels. Weak positive nuclear reaction in ZG (B), ZF (E), and ZR (H) of mature.

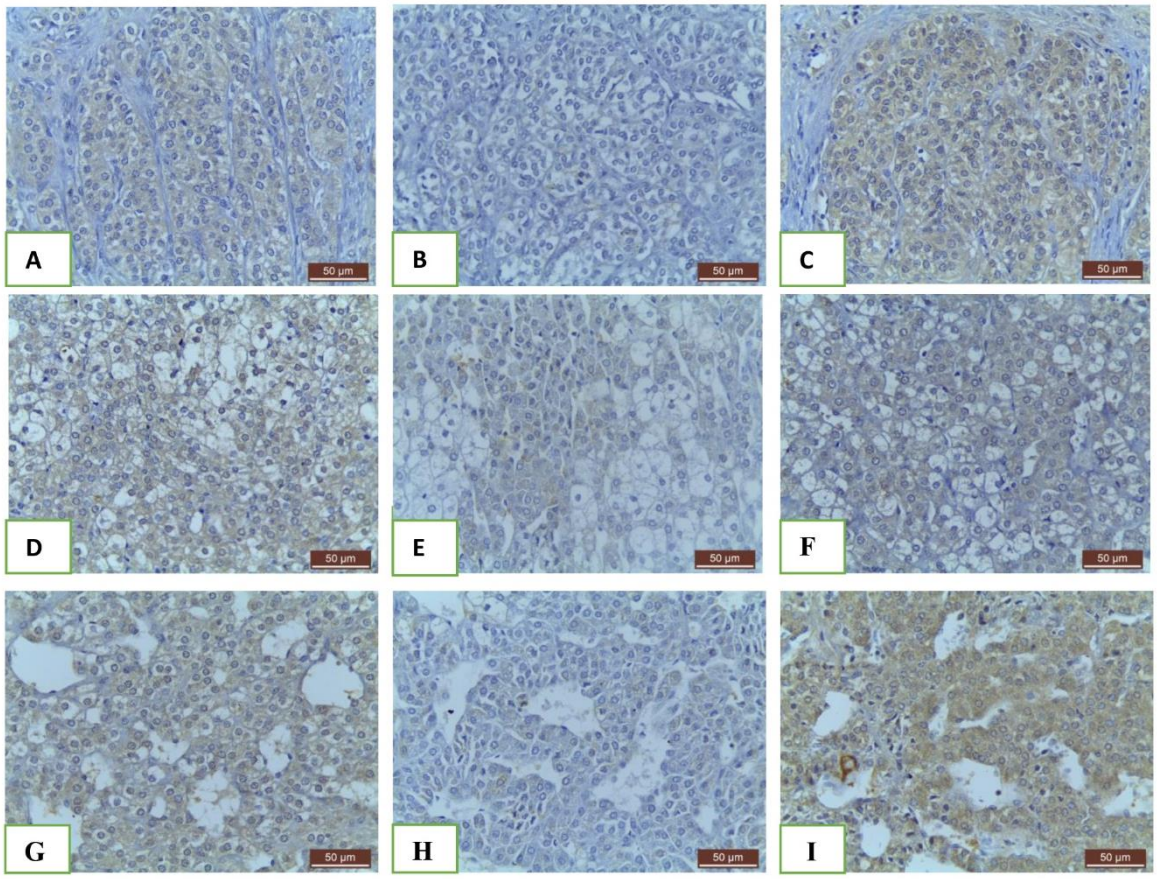

Figure 6: Photomicrograph showing immunohistochemical staining in the adrenal cortex using caspase 3. Strong positive cytoplasmic and nuclear reaction in ZG (A), ZF (D), and ZR (G) of immature, as well as in ZG (C), ZF (F), and ZR (I) of senile camels. Weak positive cytoplasmic and nuclear reaction in ZG (B), ZF (E), and ZR (H) of mature.

4. DISCUSSION

Our studies revealed that the camel adrenal glands are surrounded by thick capsules which consist of two-part structure: an outer fibrous portion and an inner cellular portion. These results agree with corresponding results of cattle (Igbokwe and Jacobs, 2016). However, these results disagreed with Ye et al. (2017) who illustrated that the adrenal capsule consists of outer, middle, and inner layer in their study on Bactrian camels. Furthermore, our study revealed the presence of collagen fibers in the capsule and parenchyma of the adrenal gland and projecting trabeculae. These findings are comparable with the observations made by Nama et al. (2015) in their study on sheep adrenal glands, providing support for the observed camel adrenal gland capsule structure. In senile animals, there was a significant decrease in the thickness of the connective tissue capsule and a significant increase in the area percentage of fibrous tissue compared to immature animals. These findings indicate age-related changes in the structural composition of the adrenal gland. The observed decrease in capsule thickness, possibly reflecting alterations in the extracellular matrix and overall tissue remodeling with aging. Similarly, the higher fibrous tissue area percentage suggests an age-related deposition of fibrous components within the gland, which may contribute to changes in its architecture and function during the senile stage .

Histologically, the adrenal cortex can be subdivided nto the zona glomerulosa, zona fasciculata, and zona reticularis. This organization of the adrenal cortex into different zones has been reported in various domestic animals, as goats in Hussein (2019) study. In immature adrenal glands of camels, the zona glomerulosa is characterized by large polyhedral cells arranged in balls or small masses beneath the developing capsule. The arrangement of the zona glomerulosa, with its small masses of eosinophilic cells resembling follicles, agrees with the observations made by Igbokwe and Jacobs (2016) on cattle. While in mature and senile camel adrenal glands, a notable characteristic is thick trabeculae extending from the adrenal capsule into the cortex to varying degrees, but they do not extend beyond the zona fasciculata. This unique feature imparts a distinct arrangement to the glomerulosa region. The trabeculae form an arching system, where the limbs of the arches divide the units of the glomerulosa into groups that exhibit significant variations in terms of number and size. This histological observation communicates with the observations made by Sheikhian et al. (2015) in their study on guinea pig and hamster .

The zona fasciculata in camel adrenal glands occupies a significant cortex area. The cells within this zone are arranged in radiating columns and exhibit a cuboid shape. Additionally, sinusoidal vessels can be observed within this region. These findings are comparable with the results reported by Hussein (2019) in their study on the adrenal glands of goats. In the camel adrenal glands, the zona fasciculata and zona reticularis exhibited a rich presence of sinusoids, some of which are notably large. This observation aligns with the findings reported by Barszcz et al. (2016) on European bison, further supporting the presence of abundant sinusoidal structures in the zona fasciculata and zona reticularis of camel adrenal glands. A broad range of steroid hormones are synthesized by the three layers of the adrenal cortex, including glucocorticoids, mineralocorticoids, and accessory sex hormones. Lack of normal adrenal gland function can disrupt electrolyte levels and carbohydrate metabolism, potentially leading to circulatory collapse, hypoglycemic coma, and even death. This has been highlighted in studies conducted by Kampan and Fluck (2008), McNicol (2008) and Ishimoto and Jaffe (2011)

Apoptosis, or programmed cell death, is a regulatory process essential for maintaining hemostasis during normal growth and development. It protects against infectious and immunological diseases (Young and Gallie, 2000). Various markers of apoptosis, including caspase 3, have been utilized to detect apoptotic cells in adrenals (Bernini et al., 2002; Yu et al., 2012 and Pereira et al., 2019). The presence of caspase-3 staining indicates the activation of apoptotic pathways and the initiation of programmed cell death. Specific studies on caspase expression in camels are lacking, research on mature camel meat quality has shown that physiological changes occur with aging, possibly related to cellular degradation or death (Suliman et al., 2013). This could explain the higher proportion of caspase 3positive reactions in senile camels compared to mature Ones. The lower expression of Caspase in mature camels compared to immature and senile camels may be attributed to a complex interplay of factors. In the immature ones, higher caspase levels might be required for developmental processes, cellular remodeling, and immune response (Zhao et al., 2016 and Liu et al., 2019). Hormonal regulation, metabolic factors, and genetic control might also contribute to these variations (Li et al., 2022 and Münz et al., 2022). As the mature camels, the need for extensive apoptosis may decrease, leading to reduced Caspase expression

Cell proliferation is a fundamental process characterized by cell division and growth, essential for producing two daughter cells and maintaining cellular homeostasis. However, abnormal cell proliferation can lead to the development of cancer (Van-Diest et al., 1998 and Grewal and Edgar, 2003). Proliferating Cell Nuclear Antigen (PCNA) is a DNA clamp protein that interacts with DNA polymerase delta in eukaryotic cells, playing a crucial role in DNA replication and repair processes (Moldovan et al., 2007). PCNA is expressed in the nucleus during the cell cycle, indicating DNA condensation and cell viability (Leonardi et al., 1992). In the adrenal gland cells of camels, immunoreactivity to PCNA can be observed in immunohistochemical staining. The positive reaction can be further categorized as strong or weak reactions. A strong positive reaction indicates the condensation of DNA strands and a higher level of cellular viability, whereas a weak reaction suggests low condensation of DNA strands (Leonardi et al., 1992) .

As in various mammalian species, including camels, we found that PCNA may exhibit a positive reaction across all age groups. However, the expression levels were likely to be higher in immature and senile individuals compared to mature ones. Studies on other species, such as viscachas, have shown variations in PCNA expression during different life stages, influenced by factors such as hormones (Rosales et al., 2016).The elevated expression in younger animals could be attributed to the increased requirements for cell growth and proliferation, essential processes for development and growth (Keim et al., 1990 and Turka et al., 1993). As the mature camels, the need for cell growth may decrease, leading to reduced PCNA expression .

5. CONCLUSION

In conclusion, the adrenal gland of camels is encircled by thick capsules which decrease in thickness with ageing. Unlike the proportion of fibrous tissue area increased in senile camels compared to mature and immature ones. The cells of ZG in the mature and senile camels show an arching

system but in immature ones, the cells are arranged in small masses that resemble follicles. The cells of ZF are arranged around large blood sinusoid in senile ages which become wider in ZR of mature camels. Immunohistochemical staining showed decrease in PCNA response in mature camels compared to immature ones and decrease in caspase 3 response in mature camels compared to immature and senile ones.

6. REFERENCES

1. Al-Bagdadi, F.A.K., 1969. The Adrenal Gland of the Camel (Camelus dromedarius): A study of the Comparative Anatomy and Lipoids. Zentralblatt für Veterinärmedizin R. A .16: 354–364.
2. Anderson, D.E., Grubb, T., Silveira, F., 1999. The Effect of Short Duration Transportation on Serum Cortisol Response in Alpacas (Llama pacos). Vet. J., 1571: 89–191.
3. Barszcz, K., Przespolewska, H., Olbrych, K., Czopowicz, M., Klećkowska-Nawrot, J., Goździewska-Harłajczuk, K., Kupczyńska, M., 2016. The morphology of the adrenal gland in the European bison (Bison bonasus). BMC Vet. Res., 12: 1–11.
4. Boenisch, T., 2009. Immunohistochemical Staining Methods. Dako, Educ. Guid.
5. Dahlborn, K., Benlamlih, S., Zine-Filali, R., Gueroulali, A., Hossaini-Hilali, J., Oukessou, M., 1992. Food deprivation and refeeding in the camel (Camelus dromedarius). Am. J. Physiol. - Regul. Integr. Comp. Physiol., 262: 6 31-6
6. Elzoghby, E., 2015. Light and Electron microscope Studies of the Adrenal Glands of the Egyptian Geese (Alopochen aegyptiacus). Univ. Stiint. Argricole si Med. Verterinara Iasi., 53: 221–229.
7. Emam, M.A., Abughrien, B., El-Zoghby, I., 2013. Role of androgen in the clitoris of camel. Glob. Vet., 11: 225–228.
8. Fouad Attia, H., 2013. Immunohistochemical Evaluation of Bcl-2 Oncoprotein in Buffalo's Adrenal Gland. J. Cytol. Histol., 04: 167-170.
9. Grandin, T., 1997. Assessment of Stress during Handling and Transport. J. Anim. Sci., 75: 249–257.
10. Grewal, S.S., Edgar, B.A., 2003. Controlling cell division in yeast and animals: Does size matter? J. Biol., 2: 3–6.
11. Hussain, R., Sarwar Qureshi, A., 2007. Age related changes in the morphometric parameters of the heart, kidneys and adrenal glands of Nili-Ravi buffalo (Bubalus bubalis). Ital. J. Anim. Sci., 6: 995–998.
12. Hussein, H., 2019. Histological and histochemical study of adrenal gland in local Iraqi coats (Capra Aegagrus). J. Sci. Tech.,12: 2305-9346
13. Ibrahim, maha, abdelrahman, howida, Elmetwaly, H., 2017. Hormonal Profile, Antioxidant Status and Some Biochemical Parameters during Pregnancy and Periparturient Period in Dromedary She Camel. Egypt. J. Vet. Sci., 48: 81–94.
14. Igbokwe, C., Jacobs, S., 2016. Age-related Morphological Changes in the Fetal Adrenal of the White Fulani (Zebu) Cattle during the Developmental Period. J. Appl. Life Sci. Int., 7: 1–10.
15. Ishimoto, H., Jaffe, R.B., 2011. Development and function of the human fetal adrenal cortex: A key component in the feto-placental unit. Endocr. Rev., 32: 317–355.
16. Jelinek, F., Konecny, R., 2011. Adrenal Glands of Slaughtered Bulls, Heifers and Cows: A Histological Study. J. Vet. Med. Ser. C Anat. Histol. Embryol., 40: 28–34.
17. Keim, D., Hailat, N., Hodge, D., Hanash, S.M., 1990. Proliferating cell nuclear antigen expression in childhood acute leukemia. Blood. 76: 985–990.
18. Kempan, P., Fluck, C.E., 2008. Adrenal gland development and defects. Best Pract. Res. Clin. Endocrinol. Metab., 22: 77–93.
19. Khanna, N.D., Rai, A.K., Tandon, S.N., 2004. Camel Breeds of India. J. Camel Sci., 1: 8–15.
20. Kimura, N., Watanabe, T., Noshiro, T., Shizawa, S., Miura, Y., 2005. Histological grading of adrenal and extra-adrenal pheochromocytomas and relationship to prognosis: A clinicopathological analysis of 116 adrenal pheochromocytomas and 30 extra-adrenal sympathetic paragangliomas including 38 malignant tumors. Endocr. Pathol., 16: 23–32.
21. Kumar, V., Sethi, R.S., Singh, O., 2021. Histological Study on the Fibrous Architecture of the Adrenal Gland During Postnatal Development in Buffalo. Indian J. Vet. Sci. Biotechnol., 17: 59–62.
22. Leonardi, E., Girlando, S., Serio, G., Mauri, F. a, Barbareschi, M., 1992. Biological Variables. J. Clin. Patho., 67: 416–419.
23. Li, F., Sun, H., Lin, X., Li, Q., Zhao, D., Cheng, Z., Liu, J., and Fan, Q. (2022). Increased cytochrome C threonine 50 phosphorylation in aging heart as a novel defensive signaling against hypoxia/reoxygenation induced apoptosis. Aging, 14(14): 5699–5709.
24. Liu, D., Wu, L., Wu, Y., Wei, X., Wang, W., Zhang, S., Yi, M., Li, J., Liu, H., and Ma, X. (2019). Heat shock factor 1-mediated transcription activation of Omi/HtrA2 induces myocardial mitochondrial apoptosis in the aging heart. Aging, 11(20): 8982–8997.
25. McNicol, A.M., 2008. A diagnostic approach to adrenal cortical lesions. Endocr. Pathol., 19: 241–251.
26. Moldovan, G.L., Pfander, B., Jentsch, S., 2007. PCNA, the Maestro of the Replication Fork. Cell. 129: 665–679.
27. Münz, S., Wolf, L., Hoelzle, L. E., Chernyakov, D., Edemir, B., and Föller, M. (2022). Impact of cytotoxic agents or apoptosis stimulants on αklotho in MDCK, NRK-52E and HK2 kidney cells. Aging. 14(18): 7282–7299.
28. Nama, K.G., Mathur, R., Dangi, A., 2015. Quantitative Histological Studies on the Parenchyma of Adrenal Glands of Marwari Sheep (Ovis aries). Indian J. Vet. Anat., 27: 9–11.
29. Pereira, S.S., Monteiro, M.P., Antonini, S.R., Pignatelli, D., 2019. Apoptosis regulation in adrenocortical carcinoma. Endocr. Connect., 8: 91–104.
30. Rehan, S., Qureshi, A.S., 2007. Morphometric analysis of heart, kidneys and adrenal glands in dromedary camel calves. J. Camel Pract. Res., 14: 27–31.
31. Riquelme, R.A., Llanos, J.A., McGarrigle, H.H.G., Sanhueza, E.M., Hanson, M.A., Giussani, D.A., 1998. Chemoreflex contribution to adrenocortical function during acute hypoxemia in the llama fetus at 0.6 to 0.7 of gestation. Endocr., 139: 2564–2570.
32. Rosales, G.J., Busolini, F.I., Mohamed, F.H., Filippa, V.P., 2016.Effects of melatonin and gonadal androgens on cell proliferation in the pituitary of viscachas (Lagostomus maximus maximus). Cell Prolif., 49: 644–653.
33. Sheikhian, A., Saadatfar, Z., Mohammadpour, A.A., 2015. A histological study of adrenal gland in guinea pig and hamster. Comp. Clin. Path., 24: 1069–1074.
34. Suliman, G.M., Hussein, E.O.S., Al-Owaimer, A.N., 2013. Improving mature camel-meat quality characteristics with calcium chloride injection. J. Camel Pract., Res. 20: 53–57.
35. Suri, S., Kour, G., Sasan, J.S., 2022. Histomorphology and Histochemistry of adrenal cortex of adult Bakerwali goat of Jammu region. J. Livest. Sci., 13: 80-87
36. Suvarna, S. K., Layton, C., & Bancroft, J. D. (2019). Theory and practice of histological techniques eighth.
37. Suzuki, T., Kachi, T., 1994. Differences between adrenaline and noradrenaline cells in cellular association with supporting cells in the adrenal medulla of the pig: an immunohistochemical study Takao. Neurosci. Lett., 3940: 217–220.
38. Turka, L. A., Gratiot-Deans, J., Keim, D., Bandukwala, R., Green, J., Strahler, J., and Hanash, S. M. (1993). Elevated proliferating cell nuclear antigen levels in immature thymocytes. Dissociation from cell cycle progression. Journal of immunology. 150(7): 2746–2752.
39. Van Diest, P.J., Brugal, G., Baak, J.P.A., 1998. Proliferation markers in tumours: Interpretation and clinical value. J. Clin. Pathol., 51: 716–724.
40. Ye, W. ling, Wang, F. ling, Wang, H. ju, Wang, J. lin, 2017. Morphology and ultrastructure of the adrenal gland in Bactrian camels (Camelus bactrianus). Tissue Cell. 49: 285–295.

41. Ye, W., Wang, F., Lv, S., Wang, Y., Dong, S., Wang, J., 2015. A Novel Path of Communication from Kidney to the Adrenal Gland in Bactrian Camels (Camelus bactrianus). Int. J. Morphol., 33: 1460–1462
42. Young, T.E., Gallie, D.R., 2000. Programmed cell death during endosperm development. Plant Mol. Biol., 44: 283–301.
43. Zaky, M., Abdel-Khalek, A., Mostafa, T., Gabr, S., Hammad, M., 2020. Productive and Reproductive Characterization, Breeding Season and Calving Season in Reference with the Effect of Parity Order on Milk Production of Camel in Egypt. J. Anim. Poult. Prod., 11: 573–581.
44. Zhao, G., Wang, H., Xu, C., Wang, P., Chen, J., Wang, P., Sun, Z., Su, Y., Wang, Z., Han, L., and Tong, T. (2016). SIRT6 delays cellular senescence by promoting p27Kip1 ubiquitin-proteasome degradation. Aging. 8(10): 2308–2323