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Comparative investigation of histological distribution of juxtaglomerular apparatus in renal parenchyma between wild (rattus norvegicus) and lab rats (rattus norvegicus)

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Abstract-The juxtaglomerular apparatus (JGA) is a specialized renal structure essential for the regulation of systemic blood pressure and glomerular filtration rate through the renin-angiotensin-aldosterone system (RAAS). It consists primarily of three cellular components: juxtaglomerular (granular) cells, macula densa, and extraglomerular mesangial (lacis) cells. with the aim of understanding the influence of environmental and physiological conditions on renal regulatory architecture. A total of 12 adult rats were used 6 wild rats captured from natural environments and 6 laboratory rats maintained under controlled conditions. Following euthanasia, kidneys were excised, fixed in 10% formalin. Tissue sections were stained using (H&E) for general morphology and Masson's trichrome for highlighting connective tissue components. Microscopic examination was conducted using light microscopy, focusing on the cortex, where JGA structures are typically located at vascular pole adjacent to glomeruli. Results showed that wild rats exhibited a significantly higher density of JGA structures compared to laboratory rats. In addition, the wild group displayed a more cellular and structurally complex macula densa region, with an increased number of juxtaglomerular cells suggesting elevated secretory activity. These differences are likely attributed to environmental and physiological adaptations. Wild rats are exposed to variable hydration, physical activity, stress, and dietary conditions that may stimulate more frequent activation of the RAAS, thereby reinforcing the development and activity of the JGA. Conversely, laboratory rats live in a controlled environment with consistent food and water availability, minimal stress, and regulated temperature, potentially reducing the physiological demand on renal regulatory systems and leading to less pronounced JGA features. Histological findings were further supported by the architectural differences observed in Masson's trichrome-stained sections, which showed more developed periglomerular connective tissue around the JGA in wild rats. These structural variations may reflect long-term adaptation to environmental stimuli.

***Key word*--- juxtaglomerular apparatus, histology, Rats.**

INTRODUCTION

Adaptations of separate mammalian species to life in diverse environments may include varying combinations of cellular, physiological, behavioral, and ecological traits (1–3). Several kidney adjustments that may be critical in enhancing water conservation are explored (4). These adaptations include alterations in the relative medullary thickness (4–6), length of the renal papilla (2), Nephron count (1,2) The proportion of long-looped nephrons and nephron heterogeneity (7) Formation of pelvic fornices (1,2), The inner stripe of the outer medulla is where collecting ducts and circulatory bundles meet. (8,9), The formation of the three medullary zones and the thin descending limb epithelium (8–10). The rat is one of the most commonly utilised study animals, notably for urinary physiology. As a model of human sickness. The time it takes for rats to eat food, which is dictated by its size, best predicts their feeding habit. Small portions of food (crumbs, flour, wheat, etc.) are devoured immediately away, whereas bigger morsels are transported to an isolated spot for uninterrupted eating. Rats avoid open locations and prefer to feed near cover, with the exception of dominating rats, who will eat food right from the source (11). The urinary system is important since it is responsible for: It regulates several physiologic activities, including red blood cell production, blood pressure and volume regulation, calcium absorption, toxin processing, and excretion. All mammalian species share basic kidney functions, as well as overall morphological and histologic structure (12). The juxtaglomerular apparatus is typically made up of granular cells produced from afferent and efferent glomerular arterioles, the macula densa, and the lacis cells. The macula densa, a specific portion of the distal tubules, has unique structural features (13), The lacis cells are organized in a pyramid, with the mesangium at the glomerular hilum at the apex and the macula densa at the base (14). The juxtaglomerular apparatus was located in the renal cortex. according to (15) is a crucial structural element of the renin-angiotensin system and one of the most important regulatory

sites for blood pressure control and renal salt and water conservation. We prepared this study in order to give a histomorphological description of the juxtaglomerular apparatus (JGA), increase its activity, and determine the extent to which the environment affects and adapts the structure of the rat kidney.

MATERIALS AND METHODS

Animal Model:

Two strain of male rats, the average of body weight was about (180g) in wild rats while in laboratory rats was about (359.3) g. The Wistar albino rats (*Rattus norvegicus*) were prepared at the animal house/pharmacology collage at Kerbala University, while the brown rat (*Rattus norvegicus*) was captured from agricultural regions and orchards in the Karbala Governorate. The rats were fed a standard diet of bread and tap water while being watched over by a specialist veterinarian. In order to provide a good and clean working environment, the right ventilation and nutrition conditions were adhered to.

Experimental design

The animals were anaesthetized with an intramuscular injection of a ketamine and xylazine combination at dosages of 80–100 mg/kg body weight and 10–12.5 mg/kg xylazine, and they were weighed using a digital balance while they were alive. They were then slaughtered at regular intervals. (16,17). In order to get access to the urine system and assess the kidneys' location, shape, and interaction with the urinary tract, the abdominal cavity was then opened and the intestine relocated. A digital camera was used to take pictures of the kidneys.

Histological preparation:

The required organs were then preserved for around 48 hours in a 10% formalin solution. kidney was sliced, preserved in 70% ethyl alcohol, dehydrated in a series of alcohol grades, cleared in xylene, and then imbedded in paraffin wax as usual. Sections of 5 µm thickness were mounted on clean glass slides using Hematoxylin and Eosin stains for a general histological analysis in accordance with (18). Masson Trichrome (MT): Collagen fibers were examined using Masson's trichrome staining (19). Measurement diameter of proximal convoluted tubule, distal convoluted tubules, bowman space and diameter of glomeruli by using ocular micrometer in light microscope.

Statistical Analysis:

Statistical analyses were conducted using SPSS version 20.0 software (SPSS® Inc., USA), with all data given as mean±SD.

Ethical approve

Under reference number, this study was carried out in the anatomical facility of the College of Veterinary Medicine at the University of Kerbala. UOK.VET.AN.2024.100.

RESULT

The renal corpuscle

The two components that make up the renal corpuscle are the Bowman's capsule and the glomerulus. Two different cell types may be found in the glomerulus, a tiny cluster of capillaries. Large fenestrae on endothelial cells prevent diaphragms from covering them. Between the capillaries are modified smooth muscle cells called mesangial cells another cell called Podocytes.

The glomerulus, a tuft-like network of tiny blood arteries (capillaries), is found at the start of a kidney nephron. The mesangium, or the area between blood vessels, is made up of intraglomerular mesangial cells and provides structural support for the tuft. The glomerulus diameter in lab rats and wild (table 1). The glomerular capillary's outside is covered in highly specialized epithelial cells called Podocytes. The cell body of Podocytes has a large number of primary, secondary, and tertiary extensions known as foot processes. The foot processes that are adjacent are joined by diaphragms with slits. Podocytes from 20 glomeruli were around 40 ± 2 in wild rats and 35 ± 2 in lab rats. Podocytes distribution and quantity are higher in wild rats than in lab rats. The inner visceral layer and the outer parietal layer were the two thin cellular layers that made up the Bowman capsule. The urinary space is continuous with the lumen of the proximal convoluted tubule, or the parietal layer is made up of a flat single layer of squamous epithelium enclosing a narrow space. The glomerular capillaries were encased in the visceral layer. The glomerular arteries and the visceral space of the renal corpuscle interact. Bowman space diameter in both wild and lab rat in (table1). The proximal convoluted tubule has a single layer of cuboidal cells with granular, eosinophilic cytoplasm and a tiny, irregular lumen. Cells were having brush border. The tube's diameter is larger in wild rats than in lab rats. In contrast to the proximal tubules, the distal convoluted tubules in the cortex had cuboidal-shaped lining cells with large, rounded nuclei and no brush border. There are fewer distal convoluted tubules in the regional cortex and they are typically shorter than the proximal convoluted tubules. There are fewer distal convoluted tubules, which may be identified by their pale cuboidal epithelial cells. It was said that these tubules have a smooth interior surface and no brush border. The diameter DCT in wild rats larger than lab rats.

The juxtaglomerular apparatus

The juxtaglomerular apparatus was positioned at the vascular pole of each glomerulus in the cortex. (figure1,2). The juxtaglomerular apparatus consisted of juxtaglomerular cells, macula densa, and mesangial cells. The nuclei of the juxtaglomerular cells were round and heavily stained with hematoxylin. In wild rats, the juxtaglomerular apparatus was considerably changed owing to the number of cells and density of macula densa, whereas in lab rats was simple and few macula densa cell. Light microscopy reveals a number of characteristics of the macula densa. When the distal tubule passes through the glomerulus, the vascular pole or hilus of the glomerulus's side has narrower, taller epithelial cells. In contrast to other tubule segments, the nuclei are located closer together and may exhibit more irregularities. When compared to laboratory rats, where the macula densa cells are grouped adjacently and seem densely stained, wild rats' macula densa cells are huge and densely

packed, making up the majority of the juxtaglomerular apparatus's total number of cells. Lacis cells are extraglomerular mesangial cells that were located in the triangle created by the macula densa and the afferent and efferent arterioles. They were composed of a collection of tiny cells (figure 3) These were less granular and had a faint stain. The tunica media's smooth muscle cells were altered at the entrance of the afferent arteriole into the renal corpuscle. There were a lot of secretory granules and not many myofilaments in the cytoplasm, and the nuclei were spherical. (figure 4).

The amount of collagen fiber in the glomerulus of wild rats is higher and more extensive than that found in lab rats. It is visible through Masson's trichrome stain and is distributed in the basement membrane of the proximal and distal convoluted tubules as well as in the capillaries inside the glomerulus (figure 5,6).

Table 1: measurement of Histological stricter in wild and lab rats as Mean and SD.

No	Wild Rats	Lab Rats
Cortex in mm	1.951 ± 0.28	3.758 ± 0.34
Medulla in mm	2.265 ± 0.23	3.484 ± 0.50
DCT in µm	31.380 ± 2.5	33.195± 6.1
PCT in µm	45.726 ± 4.20	42.404 ± 6.20
Glomeruli in µm	87.714 ± 7.46	89.907± 8.18
Bowman space in µm	3.34 ± 1.60	5.79 ± 3.01

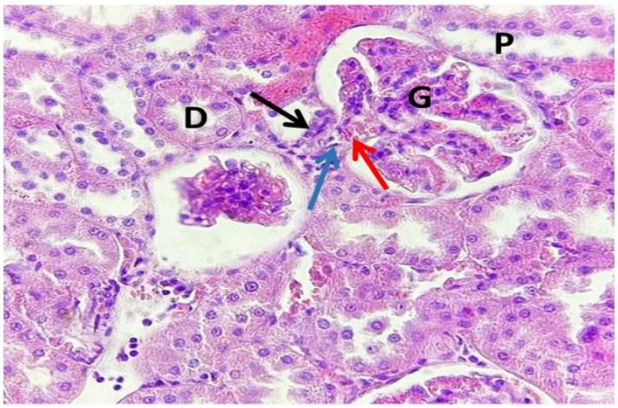

Figure 1: histological section in cortex of lab rat: showing glomeruli (G) appear Podocytes and core of blood capillary surrounded by bowman capsule the last one consist of two layer the first one called parietal layer and second one called visceral layer, proximal convoluted tubule(P) appear cuboidal cell and contain brash border , Distal convoluted tubules(D) consist of single cuboidal cell and lack the brash border , macula densa cell of juxtaglomerular apparats (black arrow), The macula densa appears multi nuclei arrangement close together but few in number. Vascular pole also appears at (red arrow) and appear afferent arteriole, lacis cell or extraglomerular mesangial cells appear triangle in shape (blue arrow). Stain with H&E stain at 400x magnification.

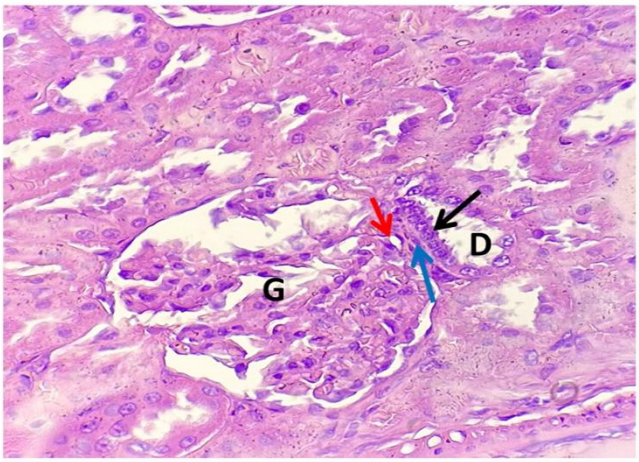

Figure 2 histological section in cortex of wild rat: showing glomeruli (G), proximal convoluted tubule(P), Distal convoluted tubules(D), macula densa cell of juxtaglomerular apparats (black arrow), The macula densa appears clearly in wild rats, and the density and number of cells are much higher when compared to laboratory rats. This is due to the adaptation to the harsh environment in which they live, as these cells work as sensors that sense the concentration of salts and dissolved materials inside the distal convoluted tubules and send signals to the other parts of juxtaglomerular apparatus. Stain with H&E stain at 400x magnification.

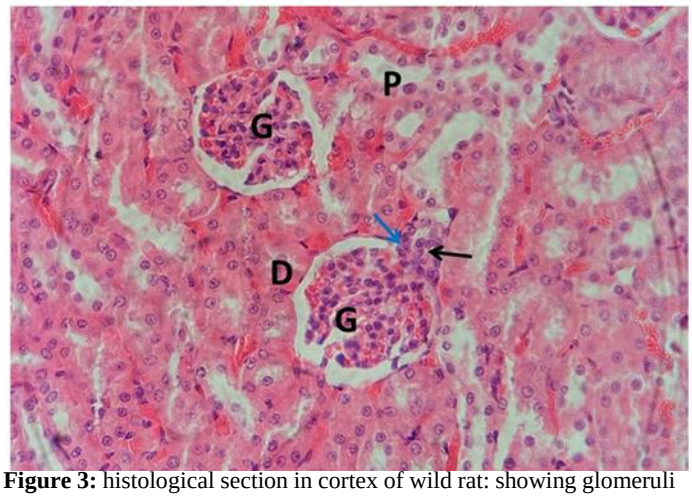

Figure 3: histological section in cortex of wild rat: showing glomeruli (G) appear Podocytes and core of blood capillary surrounded by bowman capsule the last one consist of two layer the first one called parietal layer and second one called visceral layer Distal convoluted tubules(D) consist of single cuboidal cell and lack the brash border , macula densa cell of juxtaglomerular apparats (black arrow), The macula densa appears multi nuclei arrangement close together but large in number. Vascular pole also appears at (red arrow) and *appear* afferent arteriole, lacis cell or extraglomerular mesangial cells appear triangle in shape (blue arrow). Stain with H&E stain at 400x magnification

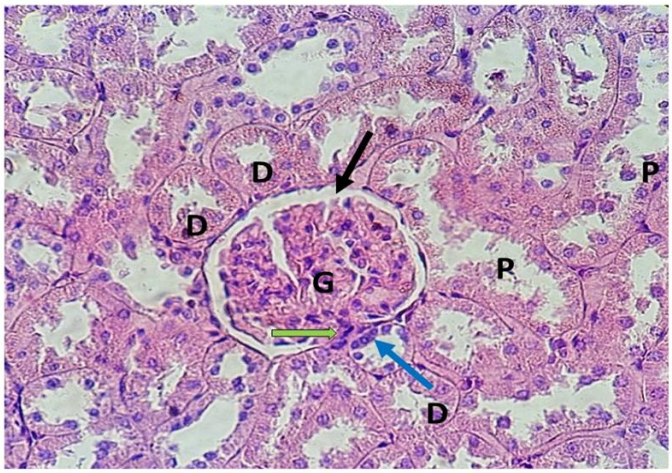

Figure 4: histological section in cortex of lab rat: showing glomeruli (G), proximal convoluted tubule(P), Distal convoluted tubules(D), macula densa cell of juxtaglomerular apparats (blue arrow), The macula densa appears clearly in lab rats, and the density and number of cells are fewer when compared to wild rats, vascular pole (green arrow). Stain with H&E stain at 400x magnification.

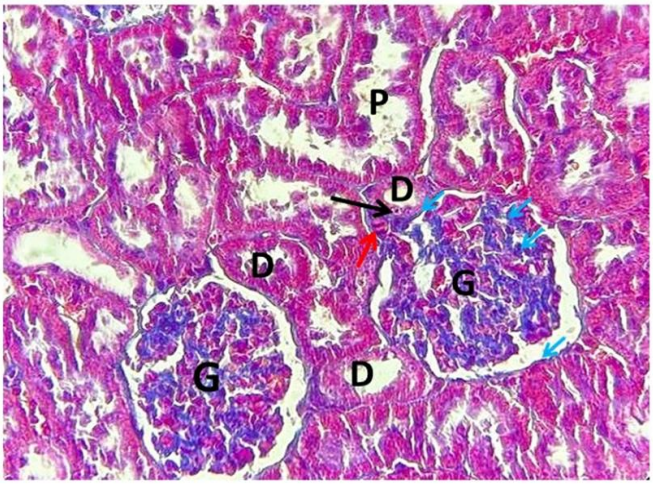

Figure 5: histological section of wild rats showing distribution of collagen fiber on basement membrane of glomeruli, distal and proximal convoluted tubules also on wall of capillary inside the glomeruli (blue arrow), vascular pole (red arrow) and macula densa is part modified from distal convoluted tubule and major part of juxtaglomerular apparatus (black arrow). Masson trichrome stain at 400x magnification.

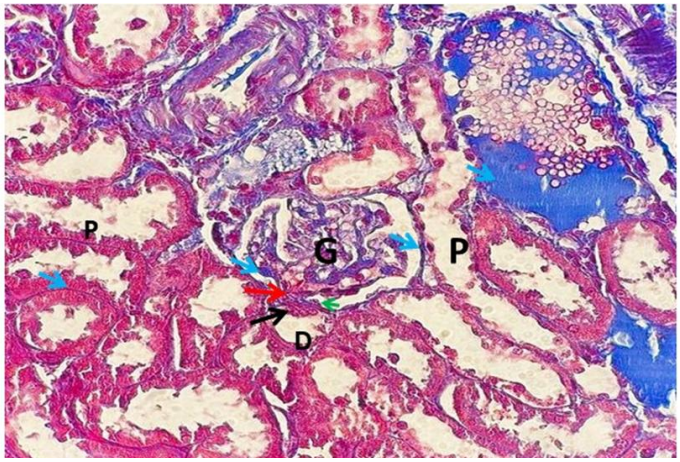

Figure 6: histological section of lab rats showing distribution of collagen fiber on basement membrane of glomeruli, distal and proximal convoluted tubules also on wall of capillary inside the glomeruli (blue arrow), on peripolar cell rest on Bowman capsule at vascular pole (green arrow), vascular pole (red arrow) and macula densa is part modified from distal convoluted tubule and major part of juxtaglomerular apparatus (black arrow). Masson trichrome stain at 400x magnification.

DISCUSSION

In the current study, two strains of rats were used, the first being the brown rat and the second being the laboratory rat, to demonstrate the tissue distribution of the juxtaglomerular system and whether environmental conditions effect on the tissue structures.

Numerous rows of glomeruli made up the cortex. This might have been brought on by the cortex's greater thickness; Merinos' libycus kidneys had shown a similar finding by (20). Wild rats' medulla length is greater than their cortex, which helps to explain how they are able to concentrate their urine due to the medulla's thickness. This finding supports numerous studies that claim that adaptations include modifications in relative medullary thickness (4–6) lead to more concentrate urine, while in lab rat the length of cortex larger than medulla because this animals live in perfect condition from available the water and food to perfect environmental conation. Endothelial cells, Podocytes, capillaries, and connective tissue make up the glomerular. Almost identical findings have been reported in the rabbit kidney by (21). Because of their exposure to dry environments and lack of water, wild rat has larger proximal convoluted tubule diameters than lab rats. This allows them to concentrate urine more than lab rats, and increasing this diameter increases the surface area of absorption, which is consistent with (22). There is no greater difference in the diameter of the distal convoluted tubules between lab and wild rats, Because the function of these tubules is only transport, and because they lack a brush border, they are unable to reabsorb, which is why their diameter is similar in wild and laboratory rats.

The juxtaglomerular apparatus position in the vascular pole of the renal glomerulus which was found in the cortex. The macula densa, mesangial cells, and juxtaglomerular cells were the parts of the juxtaglomerular apparatus that rustled agreement with results of (23). The tunica media's smooth

muscle cells, known as juxtaglomerular cells, were altered. The results showed that the nuclei were spherical agreement with results of (24) find The walls of the afferent arteriole contain specialised smooth muscles called juxtaglomerular cells, sometimes known as granular cells. When arteriolar blood pressure drops, these cells produce and release renin, which triggers the renin-angiotensin system to control blood pressure.

The macula densa is made up of several cells that were modified from distal convoluted tubules in the hilum of glomeruli, and its nuclei appeared to be close to gathering in both strains of rats. However, wild rats were more numerous than lab rats because they were exposed to harsh environments, which increased the number of these cells that sense the concentration of salts in distal convoluted tubules. These results are consistent with (15) finding The macula densa plaque is a distinct group of 15 to 20 cells located at the end of the cortical thick ascending limb that forms a juxtaglomerular apparatus-glomerular complex. These cells are critical in sensing changes in tubular fluid composition, producing and delivering signals to the juxtaglomerular apparatus, which regulates renal blood flow and GFR via tubuloglomerular feedback and renin release.

Lacis cells are extraglomerular mesangial cells composed of a cluster of tiny cells located in the triangle area formed by the afferent and efferent arterioles and the macula densa. This results. agreement with finding of (25)

The juxtaglomerular apparatus It is more advanced in wild rats than in laboratory rats, and we can see this through the density of the cells that make it up. This density is due to the role it plays in concentrating urine in animals that suffer from water shortages and arid environments.

CONCLUSION

This comparative histological study demonstrates that the distribution and morphological characteristics of the JGA are significantly influenced by environmental and physiological factors. The increased JGA development in wild rats highlights their adaptive responses to external stressors, whereas laboratory rats, despite being the standard in biomedical research, may not accurately reflect the natural variability found in wild populations. These findings underscore the importance of ecological context in interpreting renal physiology and pathology and offer valuable insights for experimental models involving blood pressure regulation and renal function.

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Conflict of interest:
The author claims that there isn't any obvious disagreement at all.

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