

## **Comparative Histological Study of Kidney in adult male squirrel (*Sciurus anomalus*) and mice (*Mus musculus*)**

**Mohammed H. Abed , Shakir M. Mirhish**

Department of Anatomy and Histology, College of Veterinary Medicine, Baghdad  
University, Iraq  
E-mail: shakirmirhish@gmail.com

### **Abstract**

Current study aimed to investigate the histological and micromorphometrical differences in the kidney of squirrel and mice. To achieve this goal, kidneys of 40 adult male squirrel and same number for mice were used in this study. The results revealed that the renal capsule of squirrel was thicker than that of mice and contain few smooth muscle fibers. The results showed that the ratio of cortex to medulla was 1:1.5 in mice and 1:2.8 in squirrel. Most of renal corpuscles of mice found in the subcapsular & midcortical regions and few corpuscles found near the corticomedullary junction, in contrast, the renal corpuscles of squirrel were distributed in mid cortical region and near corticomedullary junction, anyway, the mean diameter of juxtamedullary nephrons was higher than those of cortical and midcortical nephrons. There was a prominent variation in the diameter of bowman's space, where it was relatively wide in juxtamedullary nephrons more than that of cortical nephrons. The investigations revealed that the glomeruli in both species of animals presented three types of cells which are podocytes, endothelial cells and mesangial cells. In both species of animals, proximal tubules appeared with relatively narrow lumen and lined by simple cuboidal epithelium whereas the distal convoluted tubules have relatively a wider lumen and lined by low cuboidal cells. Thin Loop of henle lined by flattened epithelial cells with less eosinophilic cytoplasm and nuclei appeared oval-spherical in shape and bulged into the lumen whereas the thick segment appeared lined by cuboidal cells with prominent dark elongated or spherical nuclei with pinkish cytoplasm. The mean diameter of thin segment and the height of the epithelium was higher in squirrel in contrast to the diameter of thick segment which appeared higher in mice. Collecting duct characterized by clear or boundaries of their lining cells and the diameter varied between squirrel and mice and varied even with in the some species of animal. The shape of renal papilla was blunt in squirrel while it was narrow end pointed in mice as well as its length in mice was higher than that of squirrel.

**Key words:** kidney, squirrel, mice  
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## **Introduction**

Squirrels (*Sciurus anomalus*) live in almost every habitat, from tropical rainforest to semiarid desert, avoiding only the high Polar Regions and the driest of deserts. They are predominantly herbivorous, subsisting on seeds and nuts, but many will eat insects and even small vertebrates (Emamian et al., 1993; Koprowski et al., 2016). Laboratory mice are usually of the species (*Mus musculus*). They are the most commonly used mammalian research model and are used for research in genetics, psychology, medicine and other scientific disciplines, the laboratory mouse genome has been sequenced and many mouse genes have human homologues (Garner et al., 2004; Hedrich, 2004; Staats, 2010).

Urinary system is one of the vital and most important systems among an organism's body parts. It consists of Kidneys, Ureters, Urinary Bladder and Urethra. The Urinary Tract is like the plumbing system, it drains Urine from the Kidney and plays an important role in maintaining homeostasis, a set of complex processes to maintain the balance of water, ions, calcium and blood pressure, the kidneys are the most complicated part of the system (Nahon et al., 2011).

The histological and functional unit of the kidney in all animals consists mainly of renal nephrons and collecting ducts. The functional unit of the kidney can be divided into many segments (renal corpuscle, descending and ascending loop of Henle, proximal convoluted tubules PCT and distal convoluted tubules DCT (Yousif and Rabee, 2019).

Because of the paucity of studies that deal with the comparison between the urinary system of squirrel and mice, this study was conducted to investigate the histological features and micromorphometric measurements of kidney in these two animals.

## **Materials and methods**

The current study will be carried out on a total of 80 specimens. Kidneys are obtained from two species of adult healthy clinically male rodent animals, which include 40 specimens from squirrels (*Sciurus anomalus*) and the same number of specimens from mice (*Mus musculus*). All squirrels included in this study are obtained from the local market.

(Alghazzel market) in Baghdad city and then mice brought from (animal house in college vet medicine of Baghdad University).

The animals were weight alive by using digital electrical balance and the animals are euthanasia by intramuscular injection of a mixture of ketamine and xylazine at dose ketamine 25 mg/kg of body weight and xylazine 10-12.5 mg/kg (AVMA, 2013).

The kidneys were released from their fat covering connective tissue and gently removed and fixed in 10 % formalin solution after 72 hrs. and an representative specimens of 1cm<sup>3</sup> were taken. All specimens were processes by routine histological technique to obtain sections of 5-6 micrometers in thickness (Survarna et al., 2013;Sadiq and Jarjees, 2019). Hematoxyline and eosin as well as Masson's Trichrome stains were used for general histological structures and to achieve the micromorphometric measurements (Culling et al., 1985). The micromorphometric measurements include:

Thickness capsule of kidney, renal Corpuscle diameter, PCT diameter and height of epithelium, loop of henle diameter , DCT diameter and height of epithelium and collecting tubule and duct diameter( the measuring unit is/μm). All parameters were measured using the color USB 2.0 digital image camera (Scope Image 9.0- China) which was provided with image processing software. The software of camera was calibrated to all lenses of Microscope-Olympus-CX31 by aid of 0.01mm stage micrometer (ESM-11 / Japan).

### **Statistical analysis:**

Computer package (Sigma plot V12.0 / SYSTAT software) was used to conduct the histomorphometrical analysis. Data were presented as means ± SE (standard error) and were analyzed using T test with significant level set on P <0.05 (Systat, 2016).

### **Result and discussion**

Microscopic investigations of histological sections of kidney of the two species (squirrel and mice) revealed that the kidney was covered by connective tissue capsule which in turn covered by single layer of simple squamous cells or mesothelial cells. In addition, few smooth muscle cells were detected interweaving among the collagen fiber of renal capsule(Fig.1 A,B). This is in agreement with (El-Beltagy, 2002) in some small mammals and where he mentioned that each kidney is surrounded by a

capsule of connective tissue which may contain a distinct layer of smooth muscle in its deepest portion. Other researcher (Mohamed, 2014) in his study on the kidney of house mice stated that the kidney was covered by a thin dense connective tissue capsule with adipose tissue that was adherent to sub capsular connective tissue.

The data of renal capsule thickness was presented it revealed that the capsule thickness in squirrel was 3 folds more than that of mice (tab.1). This result may explain the need of squirrel kidney to such relatively thick capsule because there is a relation between the intrarenal pressure due to fluid outflow and renal capsule

Animal	Thickness of capsule	Thickness of cortex	Thickness of medulla	Ratio of cortex to medulla
Squirrel	9.1±0.39 *	1895±98.9	5286.7±84.3 *	1:2.8
mice	3.2±0.2	1950.8±56.3	2955.4±46.2	1:1.5

thickness (Mohamed, 2014).

Presence of star mean there is significant difference between squirrel and mice

In both species the medullary tissue forming a large part of renal parenchyma when compared with cortical tissue. (Fig.1-C ). this result come in agreement with results of Olukole , (2009) in African great cane rat and Ruckebuch et al., (1991) in dog and cat. In fact, the thickness of renal medulla had a clear relationship with the length of loop of henle and urine concentration.

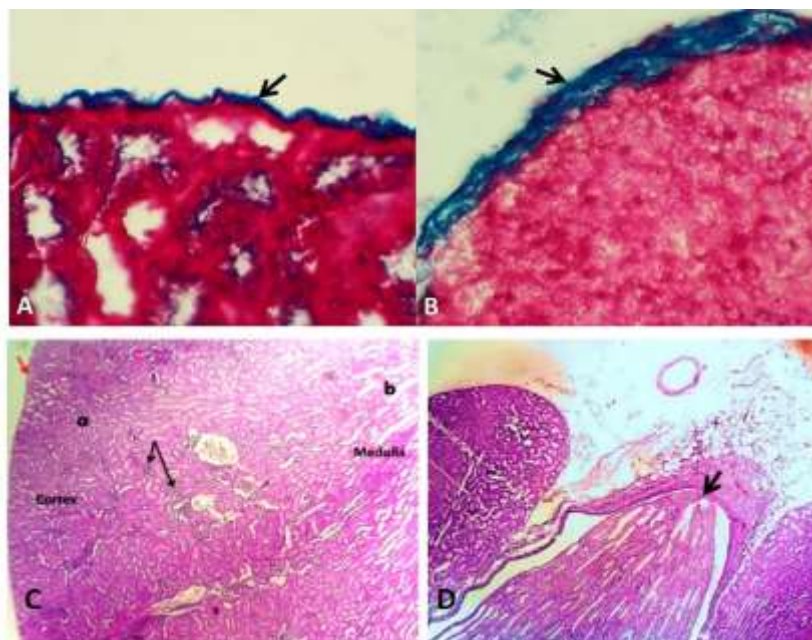


Fig.1: Histological section of kidney showing the renal capsule (black arrows) few smooth muscle cells interweaving among the collagen fiber in Mice (A) and Squirrel (B). Note the difference in thickness of capsule in the two species. Masson trichrome stain, 400X  
(C)-Histological section of renal parenchyma of squirrel consisted from two distinct areas, the outer cortex and inner medulla. Note renal capsule (red arrow), renal corpuscles (black arrows), convoluted tubules (a) and straight tubules (b). H&E, 40X.  
(D) Histological section of kidney (hilus) in mice. Note the shape of end of papilla (arrow) was pointed in mice. H&E 40X.

The cortex of kidney in both species contained renal corpuscle, proximal convoluted tubules (PCT) and Distal convoluted tubules (DCT), while the medulla contained most of the straight tubules, descends and ascends limbs of henle and collecting ducts.

The cortical thickness appeared to be similar in each of squirrel and mice ( $1895 \pm 98.9$  and  $1950.8 \pm 56.3$ ) respectively, there was no significant difference at  $P \leq 0.05$ , Whereas the medulla of squirrel appeared about 2 folds thicker than that of mice with a significant differences of  $P \leq 0.05$ , it measured ( $5286.7 \pm 84.5 \mu\text{m}$  and  $2955 \pm 46.2 \mu\text{m}$ ) in squirrels and mice respectively, The ratio of cortex to medulla was 1:1.5 in mice and 1:2.8 in squirrel (table 1).

Al-kahtani et al (2004) stated that there is a significant positive correlation between medullary thickness, urine concentration and species habitat, such correlation improves our results where we found that the medullary thickness in squirrel kidney was greater than that found in mice.

It is very clear that medullary thickness and cortico-medullary ratio play an important role in the urine-concentrating ability and renal function. Generally, the kidneys of mammals in waterless habitats have a tendency to have a relatively thicker medulla and high urine concentration ability in comparison to those of mammals in freshwater habitats (Beuchat, 1996), accordingly, our results showed that the corticomedullary ratio in squirrel appeared different significantly from that of mice where the medullary thickness and urine concentration in squirrel was higher than that of mice, these finding and interpretations improve the findings of Soleimani and Tavakolyan, (2013) in rat and Akbari et al., (2016) in Persian squirrel who stated that the kidney of the Persian squirrel has a higher urine-concentrating ability than that of the rat.

### **Nephron**

The main function unit of kidney is the nephron, which composed of renal corpuscle, PCT, loop of henle and DCT. (Fig 2,3).

The main structures that found in the cortex were the renal corpuscle, PCT, DCT and arcuate veins and arteries, while the main structures found in medulla were the straight portions (segment) of renal tubules, loop of henle and collects ducts, the structures that appeared in the hilus were the ureter, renal vein and artery embedded with in loose connective tissue and adipose tissue. These results come in agreement with Mohamed (2014) in mice and Safer et al., (1990) in desert gerbil.

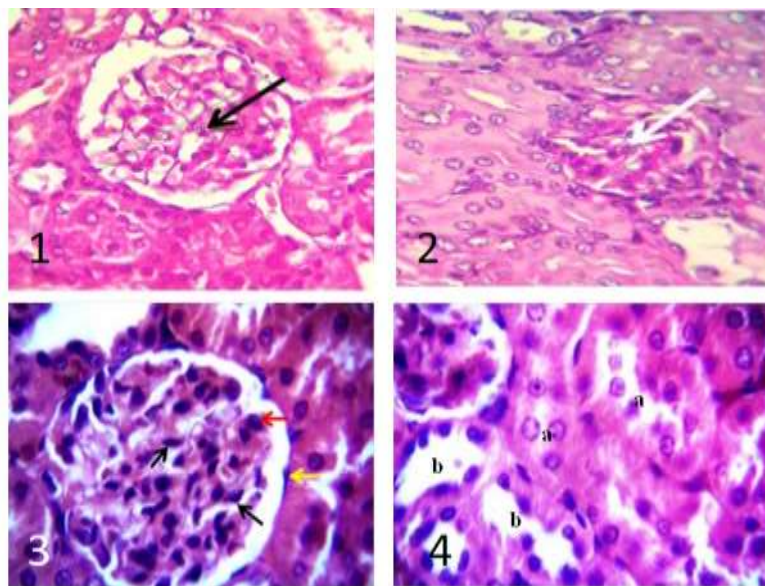


Fig.2:Histological section of kidney in squirrel (1) and mice (2). Showing the difference in diameter of Juxtamedullary corpuscles (black arrows) and cortical and midcortical corpuscles (whit arrows) H&E, 400X. (3) Histological section of kidney in mice. Showing the parietal layer of Bowman's capsule(yellow arrow), visceral layer(red arrow) podocyte and endothelium of glomerulus(black arrow) H&E, 400X. (4) Histological section of mice kidney Showing proximal tubules appeared with relatively narrow lumen and lined by simple cuboidal epithelium(a), distal tubules (b).H&E, 1000X

## Renal corpuscle

Most of renal corpuscles of mice found in the cortex (subcapsular & midcortical) and few corpuscles found near the corticomedullary junction. In contrast, the renal corpuscles of squirrel were distributed in mid cortical region and near corticomedullary junction. This study showed that the mean number of cortical nephrons were (20±0.8, 12.3±0.4) in both of mice and squirrel respectively, whereas the number of juxtamedullary nephrons were (1.4 ±0.1 and 7.1±0.3) in mice and squirrel respectively. On the other hand, the micromorphometric data showed that the mean diameter of cortical nephrons was less than that of juxtamedullary nephrons in both species (Tab.2)

**Table 2: Show the mean diameter of cortical and Juxtamedullary renal corpuscles in squirrel and mice/  $\mu\text{m}$**

Animal	diam. of corpuscle/ $\mu\text{m}$	
	Cortical	Juxtamedullary
Squirrel	65.6±2.2 *	97.1±1.8 *
mice	58.2±1.2	78.8±2.8

Presence of star mean there is significant difference between squirrel and mice

In the same manner, the mean diameter of glomeruli and bowman's space were greater in the juxtamedullary nephrons more than that of cortical nephrons in both species of animals, where the mean diameter of glomeruli of cortical nephrons was (61.1±5.6  $\mu\text{m}$ , 54.5 ±2.7  $\mu\text{m}$ ) in squirrel and mice respectively with a significant differences of  $P \leq 0.05$ . While the mean diameter of glomeruli of juxtamedullary nephrons was (85.6±5.2  $\mu\text{m}$ , 76.1±3.3  $\mu\text{m}$ ) in squirrel and mice respectively with a significant differences at  $P \leq 0.05$ . (Fig.2-1,2).

Fig 3: Histological section of kidney in squirrel (A) and mice (B). Showing the difference in diameter of Juxtamedullary corpuscles (black arrows) and cortical and midcortical corpuscles (whit arrows) H&E, 400X.

The cortical renal corpuscle appeared spherical to pear like in shape with narrow bowman's space where as the juxtamedullary nephrons appeared spherical in shape with relatively wide bowman's or urinary space.

The differences among cortical, midcortical and juxtamedullary nephrons are an important adaptation which had been observed in the kidney of desert rodent (Munkacsi and Palkovits 1977; Basuony et al.,2009). Based on the results obtained in our study, Most of renal corpuscles of mice found in the subcapsular & midcortical regions and few corpuscles found near the corticomedullary junction, in contrast, the renal corpuscles of squirrel were distributed in mid cortical region and near corticomedullary junction, anyway, the mean diameter of juxtamedullary nephrons was higher than those of cortical and midcortical nephrons.

Other researchers stated similar findings (El-Salkh et al.,2008) in his study on Egyptian desert rodents stated that in the kidney of *Psammomys obesus*, there was obvious differences in size between glomeruli and the juxtamedullary glomeruli were greater in average diameter than midcortical and superficial glomeruli. Thus good filtration in these nephrons will achieved because they have long loops, and this may result in maximal concentrating urine.

In both of squirrel and mice, the Bowman's capsule consisted from 2 layers of epithelium and a space between them called urinary or bowman's space.

The outer parietal layer made of simple squamous epithelium which appear flattened with an elongated dark nuclei. The inner visceral layer made up of specialized epithelial cells (Podocytes) which appear flattened with dark nuclei.

The visceral epithelial cell nuclei appeared large and more spherical than the endothelial cell nuclei of glomerulus but the endothelial nuclei appeared darker (fig.2-3). Approximately, similar findings had been labeled in the kidney of different Egyptian rodents by and by Safer et al., (1990) Abd-Elgawwad, (1999) in desert gerbil and El-Salkh et al., (2008).

Other important adaptation in the kidney of desert animals was an increased elongation of the inner medullary portion of the kidney of squirrel in comparison to that found in mice, such result come in agreement with the gross adaptation of desert species especially rodents (Basuony et al.,2009). Actually, this study suppose that the relative thickness of medulla was related to the habit of the animal.

There was a prominent variation in the diameter of this space. Where it was relatively wide in juxtamedullary nephrons more than that of cortical nephrons and there was a significant differences between juxtamedullary and cortical nephrons in squirrel but

there was no significances in mice. Similar findings were mentioned by Soleimani and Tavakolyan Z (2013) in Persian squirrel and rat.

### **Glomerulus**

The diameter of all glomeruli in both animals was proportioned directly with diameter of renal corpuscles. Their shape was spherical to oval with segmentation in some of them. In both species, the glomeruli looked as a mass of blood vessels but some of them appeared to be lobulated into 2-3 lobules especially in the juxtamedullary nephrons of squirrel.

These investigations revealed that the glomeruli in both species of animals presented three types of cells which are podocytes, endothelial cells and mesangial cells. The endothelial cells stained lighter than the podocytes most of glomerular capillaries contain RBCs in their lumen, the endothelial cell nuclei appeared flattened with heterochromatic stain. The podocytes nuclei found in the Bowman's space on the visceral layer of Bowman's space and looked larger with euchromatic nuclei

The urinary or Bowman's space appeared clear space between the parietal and visceral layer of Bowman's capsule. Our results showed that most of the juxtamedullary glomeruli especially in squirrel appeared larger than cortical and midcortical glomeruli in addition to the extension of loop of Henle deeply within medulla of the kidney in squirrel whereas most sections of loop of Henle found in the upper third of medulla in mice, this fact was mentioned by Michałek et al., (2016) in emu.

There was a prominent variation in the diameter of this space. Where it was relatively wide in juxtamedullary nephrons more than that of cortical nephrons and there was a significant difference at  $P \leq 0.5$  between juxtamedullary and cortical nephrons in squirrel but there was no significance in mice. (tab.2).

### **Proximal tubules PT**

PCTs are the first part of renal tubules next to the urinary pole of glomerulus and found in the cortex. The straight portion of these tubules (PCT) found in the medulla. In both species of animals, proximal tubules appeared with relatively narrow lumen and lined by simple cuboidal epithelium. Some of lining cells appeared as high cuboidal or pyramidal in shape and stained darkly with eosin or with highly eosinophilic cytoplasm (Fig.2-4). The free or luminal border of cells appeared darker

due to the presence of microvilli at the apical border. The nuclei were basally situated but in some cells were situated in the middle of cell which were spherical in shape and stained pale with hematoxylene . There was significant variation in the diameter of the proximal tubules between squirrel and mice, where the measurement showed that its diameter in squirrel was more than that of mice, but the height of epithelium in mice was higher than that of squirrel but the differences was non-significant (tab.3).

**Table 3 : Show the mean diameter and height of PCT, DCT and collecting duct in squirrel and mice/  $\mu\text{m}$**

Animal	PCT		DCT		collecting duct	
	Diameter	height of epithelium	Diameter	height of epithelium	Diameter	height of epithelium
Squirrel	40.3 $\pm$ 0.8 *	11.4 $\pm$ 0.4	30.7 $\pm$ 0.7 *	9.2 $\pm$ 0.2	75.7 $\pm$ 3.6 *	14.8 $\pm$ 0.4 *
mice	35.7 $\pm$ 0.6	12 $\pm$ 0.3	36.1 $\pm$ 1.3	9 $\pm$ 0.3	88.6 $\pm$ 4.2	12.1 $\pm$ 0.3

### Distal tubule DCT

Distal convoluted tubules also found in the cortex of kidney while the straight portion found in medulla in comparison with PCTs, their lumen appeared relatively wider and lined by low cuboidal cells. Their cytoplasm was less acidophilic and most nuclei were situated basally and stained deeply with Hematoxyline with oval or spherical shape. Histomrphometric measurements of DCTs revealed that their diameter in squirrel was less than that of mice with significant differences of  $P \leq 0.05$ . (tab.3).

### Loop of Henle

The cross sections of thin segment of loop of henle found in the medulla.it was lined by flattened epithelial cells with less eosinophilic cytoplasm. The nuclei appeared oval-spherical in shape and bulged into the lumen (Fig.3-1,2) whereas the thick segment appeared lined by cuboidal cells with prominent dark elongated or spherical nuclei with pinkish cytoplasm. the mean diameter of thin segment and the height of the epithelium was higher in squirrel in contrast to the diameter of thick segment which appeared higher in mice.

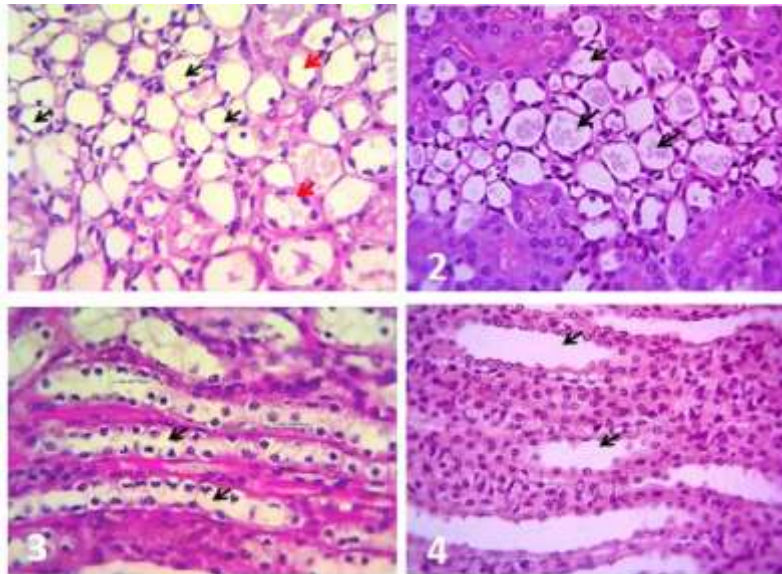


Fig.3:Histological section of squirrel kidney Showing thin segments of henle in the medulla (black arrows) and thick segment(red arrows) .H&E , 400X  
Histological section of mice kidney Showing thin segments of henle in the medulla (arrows).H&E , 400X  
Histological section in medulla of mice kidney Showing the collecting duct (arrows).H&E , 400X  
Histological section in medulla of squirrel kidney Showing the collecting duct (arrows).H&E , 400X

Our findings come in agreement with (13) in albino rats and (28) in African Grass cutter, while disagree with (29), who reported that the proximal convoluted tubules are lined with columnar epithelial but in agreement with that proximal tubule is more narrow than the distal convoluted tubule.

### Collecting duct

The collecting duct in both species of animals were found in the medulla. But some sections of the arcuate ducts were found in the deep part of the cortex. The duct was lined by cuboidal to high cuboidal to columnar epithelial cells with dark prominent spherical nuclei and clear cytoplasm. These duct characterized by clear or boundaries of their lining cells (Fig.3-3,4) the diameter of collecting ducts varied between squirrel and mice and varied even with in the some species of animal. The results showed diameter appeared to increase toward deepest part of medulla, the diameter in mice was higher than that of squirrel while the height of epithelium was higher in squirrel more than that of mice (tab.3).

This result agreement with (13,14and 29) as they noted that the epithelial cells of the collecting tubules are pale and vary from cuboidal to columnar close to the papilla and

the cell boundaries are normally clear when compared with the cells of the proximal and distal convoluted tubules in progress toward the renal papilla. The terminal portion of these tubules is lined by columnar epithelium and is called papillary ducts. The deepest part of medulla continued to the hilus as renal papilla in both species of animals containing a large number of cross sections of collecting ducts. The shape of papilla was blunt in squirrel while it was narrow end in mice as well as its length in mice was higher than that of squirrel (tab.4) (Fig.1-D).

**Table 4: Show the mean diameter of hilus , renal pelvis and Length of papillae in squirrel and mice/  $\mu\text{m}$**

Animal	Diameter of hilus	Diameter of renal pelvis	Length of papillae
Squirrel	1107.4 $\pm$ 28.9 *	2444.9 $\pm$ 54.5 *	2275.9 $\pm$ 55.9 *
mice	1342.7 $\pm$ 44.2	1513.4 $\pm$ 34.9	3263.9 $\pm$ 60.2

Actually, our results were different from that of Mohamed (2014) who described the papilla in the house mice as a single elongated renal papillae, and disagree also with El-Beltagy (2002) in albino rats and Ahmadizadeh et al, (1984) in Spiny mice, who mentions the renal papilla was markedly sharp pointed, longer and extended down into the renal pelvis while current result agreement with El-Beltagy (2002 in true desert rodents comparing to the corresponding papillae of both the hedgehogs and the guinea pigs which were relatively shorter and blunt

Renal pelvis appear to be lined with transitional epithelium with diameter of (2444.9  $\pm$  54.5)  $\mu\text{m}$  in squirrel and 1513.4  $\pm$  30.9)  $\mu\text{m}$  in mice (tab.4).

The hilus of kidney in both species of animals contain loose connective tissue and adipose connective tissue as well as the ureter, renal artery and renal vein. The diameter of hilus in mice was higher than that of squirrel (1342.7  $\pm$  44.2, 1107.4  $\pm$  28.9  $\mu\text{m}$ ) respectively. (tab.4)

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