

# Comparative Study Between Camel and Cat Kidney by Using Different Lectins

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## ABSTRACT

**Introduction:** the present study was undertaken to compare between the camel kidney with that of cat kidney. Camels are herbivorous animals while cats are carnivorous animals for this reason the difference in kidney was studied by lectins histochemistry to meet the physiological needs difference such as feeding. Samples were collected from camel and cat and processed for investigation. Lectins were studied in kidneys using concanavalin (Con A), ulex europaeus agglutinin (UEA), ricinus communis agglutinin (RCA), wheat germ agglutinin (WGA) and soybean agglutinin (SBA). Con A appeared moderate reactivity in Bowman capsule, podocytes and distal convoluted tubules (DCT) in camel kidney while cat kidney showed no reactivity in Bowman capsule, podocytes, intense in glomerular capillaries, DCT and mild in proximal convoluted tubule (PCT). UEA was moderate intensity in Bowman capsule, podocytes, DCT in camel and cat kidney. WGA was intense in Bowman capsule of camel kidney while no signal in cat kidney. RCA was showed moderate reactivity in Bowman capsule and surrounding tubules of camel kidney while cat kidney was showed intense reactivity in proximal and distal tubules. SBA showed intense signal in podocytes and moderate in surrounding tubules of camel kidney while cat kidney showed intense in Bowman capsule and moderate in surrounding tubules. Therefore, using Con A, UEA, WGA, RCA and SBA in kidney can propose that some lectins had different location in kidney and we can understand the functions of these lectins among species. We speculated that it may be critical to understand the various localization of lectins in different nephron parts of camel and cat kidney.

**Keywords:** Immunohistochemistry; Camel; Cat; Lectins; Kidney.

## Introduction

Lectins, which are proteins link capable of being reversed to particular sugars of glycoprotein have been used as inquiry for concurrence of carbohydrate molecules on the surface of the cell or in cytoplasmic organelles (Hennigar *et al.*, 1985; Spicer and Schulte, 1992 and Roth, 1996). The kidney has various structures, and lectin localization in each nephron segment providing an important data about identification of each cell's function (Faraggiana *et al.*, 1982; Hennigar *et al.*, 1985 and Truong *et al.*, 1988).

The camel is known to maintain water over physiological processes and producing extremely concentrated urine (Nielsen, 1979). The segmentation of the medulla into inner and outer region is a noticeable characteristic in relating kidney structure to the capability of an animal to form much concentrated urine (Brenner, 2019). The production of extremely concentrated urine is a significant factor in water conservation. The kidney must have definite anatomical features for an efficient counter-current system of urine concentration to produce concentrated urine.

The medullary architecture and its combination with the renal pelvis and transport properties of the nephrons propose that the anatomic relationships

of these structures may participate to urine concentration (Nawata *et al.*, 2018). The feature of renal anatomy changes in different mammals according to the drought of the habitat (Sperber, 1944).

The kidney anlage of the 11 days embryos of mouse contain the distended tip of the ureter bud and the metanephric blastema with mesenchymal cells encompassed by extracellular matrix. The mesenchymal cell surface interacted with Con A, Ricinus communis (RCA), Wheat germ agglutinin (WGA), and succinylated WGA. A prevalent, spot-like cytoplasmic Con A engaged was visible, as well as a further peripherally located WGA-binding structure, maybe identical to the Golgi apparatus (Laitinen *et al.*, 1987).

Holthofer (1983) described many lectin localizations in 14 animal species including mammal, avian, reptile and freshwater fish but no data about camel and cat. Con A distinguished mannosyl residues of glycoconjugates and Con A in adult mouse kidney had many binding patterns to kidney and signals were in all segment of nephron but binding sites were specific to proximal tubules and Con A strongly bind to glomeruli (Laitinen *et al.*, 1987).

UEA-I was fructose binding sites found in thin limbs of the loop of Henle, thick ascending limbs

and distal tubules. Apart from UEA, lectins showed heterogeneous bindings in collecting ducts in dog (Yabuki *et al.*, 2012).

WGA, recognizing both sialic acid and N-acetyl glucosaminyl moieties and binds to all parts of the nephron over the cell membranes and the basement membranes, In addition to interstitial cells and endothelia of glomerulus. The glomerular podocytes surface and the brush border of the proximal tubules straight part or third segment display farthest notable positivity in adult mouse kidney. Neuraminidase processing of the sections abolishes surface positivity from podocytes, showing that sialic acids were responsible for these reactivities in adult mouse kidney (Goldstein and Hayes, 1978; Monsigny *et al.*, 1979).

Since sialic acid is the major receptor for WGA in kidneys (Holthofer *et al.*, 1981) and sialic acids are major components of the glomerular polyanions, which are primary for the normal glomerular filtration (Latta, 1980; Reeves *et al.*, 1980). WGA consequently seems to be a marker for the glomerular filtration barrier in all the species (Holthofer, 1983).

Hawthorne *et al.* (1986) reported a collection of WGA-positive substance in the glomerular basement membrane and mesangium in diabetic rats. In addition, Yonezawa *et al.* (1986) reported that some lectin had engaged sites in the sclerotic areas of glomeruli in diabetic mice. Aguirre *et al.*, (1993) found that some lectins increased in the kidney of diabetic hamsters in the influenced bowman's capsules and glomeruli in addition to degenerated epithelial cells of renal tubules. This suggests that glycoconpounds accumulated in such affected renal structures.

RCA-I binds preferentially to oligosaccharide structures terminated in galactose however may also interact with N-acetyl galactosamine (Debray *et al.*, 1981). It binds hardly to the brush border and cytoplasmic granules of the proximal tubules and also reacts diffusely with basement membranes, cell surfaces and endothelial cells. The luminal surface of the distal convoluted tubules does not react with RCA-I in adult mouse kidney (Hayes and Goldstein, 1974; Lotan *et al.*, 1975). On maturation on days 15 to 17 of mouse kidney, RCA reactivity in the mesangium and inner parts of the glomerular capillary wall were also distinguished during the capillary loop stage but later decreased (Laitinen *et al.*, 1987).

RCA-I shows heterogeneous binding in the walls of the capillary glomeruli in canine kidney (Yabuki *et al.*, 2012). RCA-I in the kidney of the rat (Laitinen *et al.*, 1989) showed reactivity in embryonic glomeruli stronger than that in adult glomeruli, with highly more reactivity in the developing structures. Close connection between RCA-I reactivity and the degree of glomerular injury was indicated in a murine model of glomerulonephritis (Kizaki *et al.*, 1989).

Holthofer (1983) reported that SBA which is N-acetylgalactosamine specific were found in the

proximal tubule in pigs, rats and humans, while SBA signals were specific in the distal tubule in guinea pigs, rabbits, mice and rat. SBA did not localize in the glomeruli of canine kidney; so, it was explained that galactose residues were heterogeneous in distribution and present in canine glomeruli (Yabuki *et al.*, 2012).

There are no any studies described lectins localization in camel and cat and in our present study; we used five different lectins to understand the function of each lectins and also roles of each nephron segments of the two different species.

## Materials and Methods

### Animals and tissue preparation

All experiments were performed in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals and the protocols accepted by the Ethics Animal Care Committee of Damanhour University, Egypt (Approval No. 24102020).

The current study was carried out on a total of 15 a healthy male Egyptian camel kidney (*Camelus dromedarius*) of age ranges from 10-15 years age. Camels were slaughtered in the Kom Hamada camel slaughterhouse (Elbehira governorate, Egypt), following the normal abattoir procedures. Kidney immediately after the camels were slaughtered, two tissue specimens were collected from each kidney of the slaughtered camel.

The samples were collected from domestic male cats (stray or feral cats) 15 healthy animals of age ranges from 3 to 4 years. Cats were obtained at laboratory of the histopathology of veterinary medicine Damanhour university, Egypt. Following ether inhalation, the animals were euthanized by cervical dislocation. Kidneys with abnormal shapes hemorrhage or gross pathological signs were excluded. Then the specimen was immediately fixed in 4% paraformaldehyde dissolved in 0.1 phosphate-buffered saline (PBS) for 48 h for histopathological and immunohistochemical evaluation.

### Light microscopic examination

The kidney samples were dehydrated in ascending grades of ethyl alcohol starting from 50% to absolute one. The clearance of the samples was applied using xylene (three changes) and then paraffin impregnation was done in the hot oven using melted paraffin wax (three changes) at 56°C. Finally blocks of the processed samples were prepared using paraffin wax and cut using rotatory microtome. Thin paraffin sections (5-7 µm-thick) were cut from the samples' blocks and mounted on egg albumin-glycerin coated glass slides and dried in an electrical incubator for 30-60 minutes at 45°C then stained with the following stains according to Bancroft and Layton (2013).

1. Hematoxylin and eosin (H and E) for general inspection of the organ.

2. Periodic Acid Schiff reaction (PAS) for the detection of the neutral mucopolysaccharides.

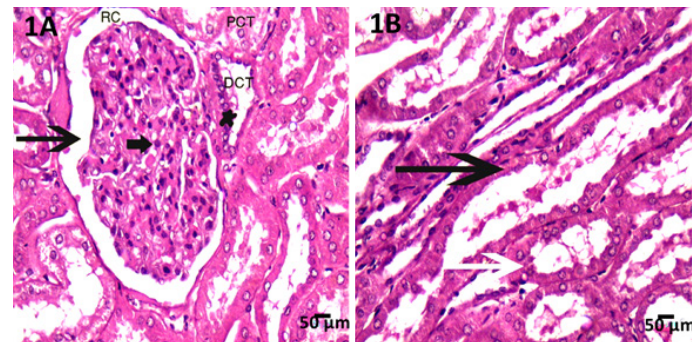
**Immunohistochemistry For lectins staining:**

4 µm-thick paraffin sections were prepared, deparaffinized by xylene, rehydrated in descending grades of ethyl alcohol and washed by distilled water. Later on the prepared slides were stained for lectins histochemistry at Medical Research Institute, Alexandria University, Alexandria; Egypt, as described below.

Antigen retrieval was done as a first step by 0.05% trypsin at 37°C for 2 min followed by washing with distilled water. Deactivation of endogenous peroxidase by 0.3% H<sub>2</sub>O<sub>2</sub> in Methanol for 20 min was done second. After washing with phosphate buffer saline (PBS), the nonspecific reaction was blocked with 1% Bovine Serum Albumin (BSA) (Vector Laboratories, USA) in PBS for 60 min at room temperature was the third step. Then the biotinylated lectins were incubated at 4°C overnight. After washing with PBS (three changes, 5 min each), ABC (Avidin-Biotin complex) solution was applied at the room temperature for 30 min. After washing with PBS (three changes, 5 min each), diaminobenzidine solution (DAB) was applied for 4 min for color development. The reaction was stopped by distilled water and the sections were counterstained by Mayer's hematoxylin.

corpuses in the cortex are proximal convoluted tubules which lined by high cuboidal epithelium and have narrow lumen obscured by microvilli.

The distal convoluted tubule of camel was lined by low cuboidal epithelium and has wide lumen with ill-developed microvilli. In the wall of distal convoluted tubules there is juxta glomerular apparatus as the afferent arterioles become contact with the distal tubule stained by hematoxyline and eosin (Fig. 1A). The medulla of camel had large collecting ducts which lined by high cuboidal epithelium and collecting tubules which lined by low cuboidal epithelium (Fig. 1B).



**Figure 1.** Light micrograph of camel kidney. 1A: The cortex with renal corpuscle (RC) surrounded by wide bowman's space (long arrow) and podocytes cells (short arrow), distal convoluted tubules (DCT) at juxtaglomerular apparatus (asterisk). x40. 1B: Showing medulla with collecting ducts (arrows) which lined by cuboidal. X40. Scale bar =50 µm. H&E.

**Table 1.** List of lectins used in the current study

| Lectins group                     |       | Name                  | Sugar binding specificity | Concentration | Binding | Inhibitor sugar |
|-----------------------------------|-------|-----------------------|---------------------------|---------------|---------|-----------------|
| Glucose binding lectins           | Con A | Concanavalin A        | α-D-Man<br>α-D-Glc        | 10µ/ml        | HRP     | Man             |
| Fructose binding lectin           | UEA   | Ulex europaeus        | α-L-Fuc                   | 20µ/ml        | HRP     | Fuc             |
| Glucosamine binding               | WGA   | Wheat germ agglutinin | β-D-GlcNAC                | 5µ/ml         | HRP     | GlcNAC          |
| Galactose binding lectins         | RCA   | Ricinus communis      | Gal β1-4GlcNAC            | 5µ/ml         | HRP     | Gal             |
| Galacose, N-Acetylgalactose amine | SBA   | Soybean Agglutinin    | GalNACα1-O-Se             | 20µ/ml        | HRP     | GalNAC          |

Abbreviations used: GalNAC, N acetyl galactosamine; GlcNAC, N-acetyl glucosamine; gal, galactose; Man, mannose; α-D-Man, Alpha-linked mannose; α-D-Glc, Alpha-linked glucose; Fuc, fucose, HRP, Horse radish peroxidase.

**Results**

**Anatomical and histological structures of Camel and cat Kidneys**

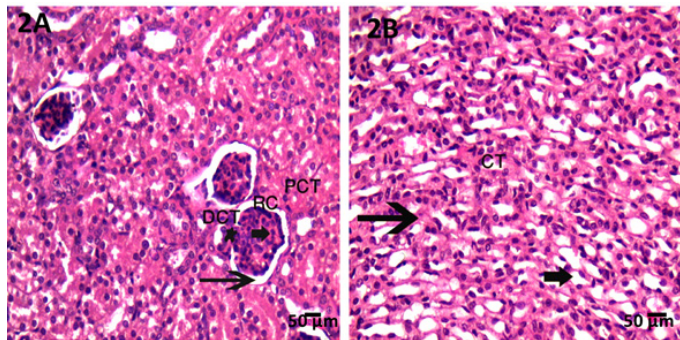
The adult camel kidneys have well developed cortex and medulla. The cortex showed mature nephrons with large renal corpuscle which consists of tough of capillaries (glomerulus) and visceral epithelium (podocytes cells), the renal corpuscle lined from outside by parietal epithelium (simple squamous epithelium). There is wide space between the visceral and parietal epithelium was the bowman's space which the filtrate collected in it. The renal tubules surrounded the renal

Structure of cat kidney similar to the histological structure of camel kidney except the cortex was showed small renal corpuscle with narrow bowman's space, the cortex crowded by smaller renal tubules (PCTs, DCTs) and also the juxtaglomerular apparatus was seen in wall of distal convoluted tubules (Fig. 2A). The medulla was showed collecting ducts and tubules (Fig. 2B).

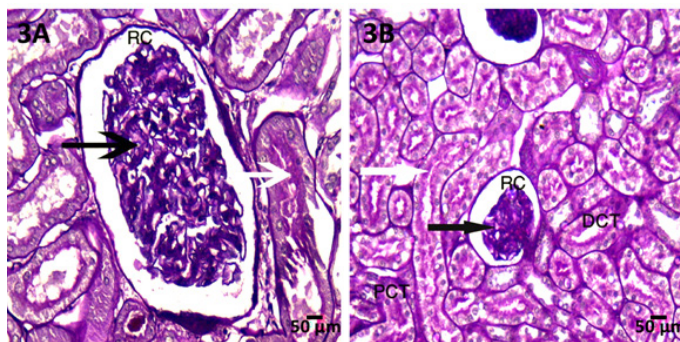
The camel kidney was showed well developed glomerular basement membrane, the proximal convoluted tubules showed well developed brush border and distal convoluted tubules showed ill-

developed microvilli by PAS stain (Fig. 3A). The cat kidney showed positive reaction by PAS stain and brush border of PCT and mild reaction in apical surface of DCT (Fig. 3B).

The Lectin binding activity was studied in our research by using Con A, UEA, WGA, RCA and SBA.



**Figure 2.** Light micrograph of cat kidney. 2A: Showing the cortex with renal corpuscle (RC) which is surrounded by Bowman's space (thick arrow), proximal convoluted tubules (PCT), distal convoluted tubules (DCT) which show the juxtaglomerular apparatus in the wall (asterisk). 2B: Showing medulla with collecting tubules and ducts (arrows). X40. Scale bar=50 µm. H&E.



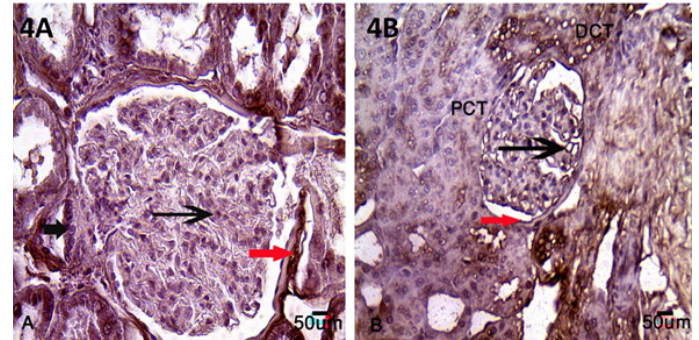
**Figure 3.** Photomicrograph of kidney PAS staining. 3A: Camel kidney showing RC with positive reaction in glomerular basement membrane (black arrow) and apical microvilli of renal tubules (white arrow). 3B: Cat kidney showing renal cortex with positive reaction in RC in glomerular basement membrane (black arrow) and well-developed microvilli in PCT (white arrow), DCT. X40. Scale bar=50 µm. PAS stain.

**The Concanavalin (Con A) in camel and cat kidneys**

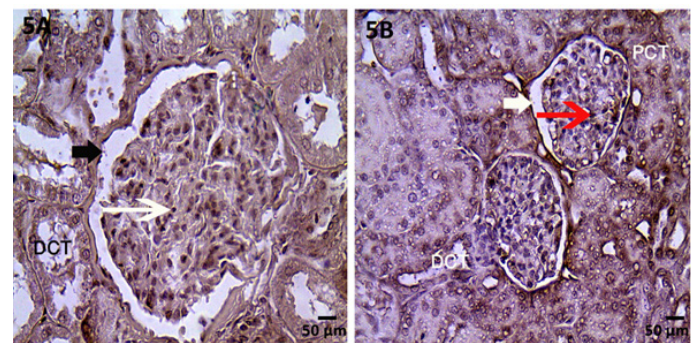
Glucose binding lectins showed moderate reactivity in camel kidney cortex in podocytes cells in renal corpuscle as well as Bowman's capsule and surrounding distal convoluted tubules renal which showed juxtaglomerular apparatus in its wall when come in contact with afferent arterioles at renal corpuscle (Fig. 4A). The cat kidney stained by Con A was showed intense reactivity in glomerular capillaries of renal corpuscle and DCT while it showed mild in apical microvilli of proximal convoluted tubules and Bowman's capsule (Fig. 4B).

**Ulex europaeus agglutinin (UEA) in camel and cat kidneys**

Fructose binding lectin showed moderate reactivity in podocytes cell in renal corpuscle of camel kidney and Bowman's capsule while mild in DCT (Fig. 5A). Cat kidney showed also moderate reactivity in podocytes cells as well as Bowman's capsule and surrounding renal tubules PCT and DCT (Fig. 5B).



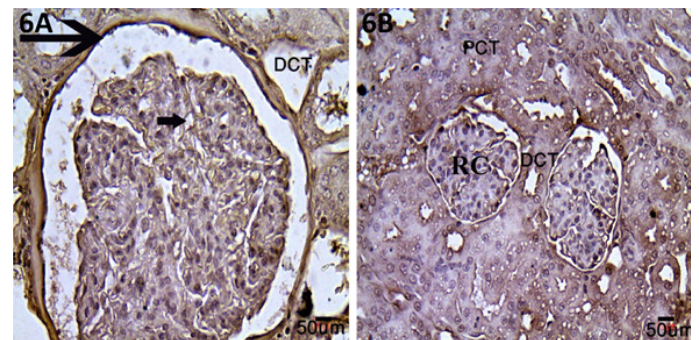
**Figure 4.** Photomicrograph showing ConA reactivity. 4A: Camel kidney showing moderate Con A reactivity in renal corpuscle in podocyte cell (long arrow), Bowman's capsule (red arrow) and DCT which show juxtaglomerular apparatus (short arrow). 4B: Cat kidney with intense Con A reactivity in glomerular capillaries (arrow) and distal convoluted tubules (DCT) while mild in apical surface of PCT and Bowman's capsule (red arrow). X40. Scale bar=50 µm.



**Figure 5.** Photomicrograph showing UEA reactivity. 5A: camel kidney with moderate reaction in RC especially podocytes cells (arrow) and Bowman's capsule (short arrow) while mild reaction in DCT. X40. 5B: Cat kidney with moderate reaction in RC in podocytes cells (arrow), Bowman's capsule (short arrow) and surrounding renal tubules (DCT), proximal convoluted tubules (PCT). X40. Scale bar=50 µm.

**Wheat germ agglutinin (WGA) in camel and cat kidneys**

Glucosamine binding lectin of camel kidney showed mild reactivity in glomerular capillaries, intense in parietal epithelium of (Bowman's capsule) of renal corpuscle and moderate in DCT (Fig. 6A) while cat kidney showed mild reactivity in DCT, PCT and no reaction in renal corpuscle (Fig. 6B).

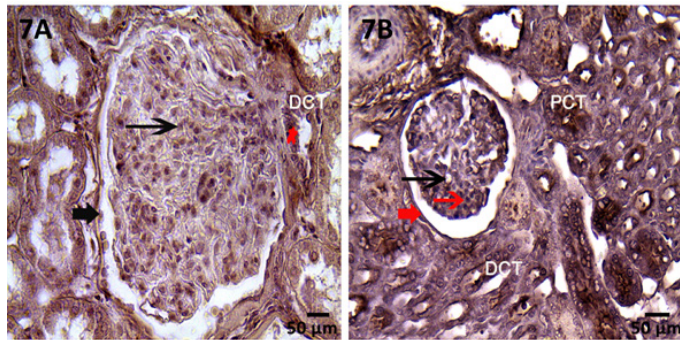


**Figure 6.** Photomicrograph showing WGA reactivity. 6A: Camel kidney with mild WGA reactivity in glomerular capillaries (short arrow), intense in parietal epithelium (long arrow) and moderate in DCT (long arrow). 6B: Cat kidney with moderate reactivity in DCT and PCT while no reactivity in RC. X40. Scale bar=50 µm.

**Ricinus communis (RCA) in camel and cat kidneys**

Galactose binding lectins, camel kidney showed moderate reactivity in podocyte cell in renal corpuscle,

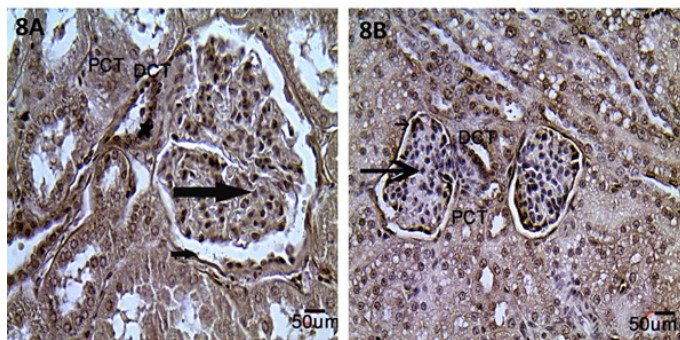
bowman's capsule and DCT with juxtaglomerular apparatus (Fig. 7A) while cat kidney showed mild reaction in glomerular capillaries, bowman's capsule while moderate in podocyte, intense reaction in DCT and PCT (Fig. 7B).



**Figure 7.** Photomicrograph showing galactose binding lectin (RCA). 7A: Camel kidney with moderate reactivity in podocyte cell (long arrow), Bowman's capsule (short arrow) and DCT with juxtaglomerular apparatus (asterisk). 7B: Cat kidney with mild reaction in glomerular capillaries (arrow), Bowman's capsule, moderate in podocytes (red arrow), intense reaction in DCT and PCT. X40. Scale bar=50 µm.

**Soybean Agglutinin (SBA) in camel and cat kidneys**

Galucose, N-Acetylgalactose amine in camel kidney showed intense reactivity in Podocytes cells, DCT with juxaglomerular apparatus while moderate Bowman's capsule and PCT (Fig. 8A). The cat kidney showed moderate reactivity in podocytes and DCT, intense in Bowman's capsule while mild reactivity in PCT (Fig. 8B).



**Figure 8.** Photomicrograph showing SBA reactivity. 8A: camel kidney showing SBA with intense reactivity in Podocytes cells (arrow), DCT with juxtaglomerular apparatus (short arrow) while moderate in PCT and Bowman's capsule (short arrow). 8B: Cat kidney with moderate reactivity in podocytes (arrow), DCT, intense in Bowman's capsule (short arrow) and mild in PCT. X40. Scale bar=50 µm.

**Table 2.** Lectin binding activity in camel and cat kidney

| Biding sites                  | Con A |      | UEA   |     | WGA   |     | RCA   |      | SBA   |      |
|-------------------------------|-------|------|-------|-----|-------|-----|-------|------|-------|------|
|                               | Camel | Cat  | Camel | Cat | Camel | Cat | Camel | Cat  | Camel | Cat  |
| <b>Bowman's capsule</b>       | +++   | -    | +++   | +++ | ++++  | -   | +++   | ++   | +++   | ++++ |
| <b>Podocytes</b>              | +++   | -    | +++   | +++ | -     | -   | +++   | +++  | ++++  | +++  |
| <b>Glomerular capillaries</b> | -     | ++++ | -     | -   | ++    | -   | -     | ++   | -     | -    |
| <b>PCT</b>                    | -     | ++   | -     | +++ | -     | +++ | -     | ++++ | +++   | ++   |
| <b>DCT</b>                    | +++   | ++++ | ++    | +++ | +++   | +++ | +++   | ++++ | ++    | ++   |

PCT, Proximal Convolved Tubules; DCT, Distal Convolved Tubules; Reactivity: -, +, ++, +++, +++++ denote negative, weak, mild, moderate and intense, respectively.

**Discussion**

We observed five different lectins patterns in two different species (healthy camel and cat kidney) and in our knowledge, there are no any studies or researches described lectin binding sites of kidney in these two animals.

We found that there are many lectin patterns between species as Laitinen et al. (1987) showed that Con A had intense expression in mouse PCT and glomeruli while in our study in camel, there is no any signal in PCT or glomeruli but in cat showed intense signals in glomeruli and DCT.

Yabuki et al. (2012) showed that in dog; Con A weakly stained capillary endothelial cells in glomeruli, mild to moderate signals in Bowman capsules. Positive signals were observed in the cytoplasm of PCTs and DCTs showed granular positive signals. On the other hand, Con A of cat kidney showed intense reactivity in glomerular capillaries of renal corpuscle and DCT, mild in PCT and Bowman's capsule. Our study demonstrated that Con A signals were higher in cat than dog.

WGA binding to capillary endothelium of glomeruli had been distinguished as a common characteristic in most animal species (Holthöfer, 1983). Yabuki et al. (2012) showed that WGA and RCA were localized in glomeruli and binding to the capillary walls in dog. In contrast to our study, we found that there were no lectin signals of our five lectins in glomerular capillaries of camel but in cat we found that Con A was intense in glomerular capillaries and RCA was mild. We found that in camel, WGA was intense in Bowman capsule while Con A, UEA, RCA and SBA were moderate. Our results in camel showed that however there is no lectins in glomerular capillaries in camel, but they localized in Bowman capsule of camel for urine filtration and this lectins localization might relate to water conservation of camel. WGA in cat kidney showed no reaction in renal corpuscle (Bowman capsules and glomeruli) and mild reactivity in DCT, PCT. Our study elucidated that WGA in cat lower than dog which related to glomerular filtration.

Concerning to PCT of herbivores animals, Holthofer (1983) showed that cow kidney stained by UEA and SBA were negative and faint while WGA and RCA

were moderate in PCT. In other hand, our study in camel showed that UEA and SBA were negative and moderate while WGA and RCA were negative in PCT.

Concerning to DCT of herbivores animals, Holthofer (1983) showed that WGA and SBA were intense while UEA and RCA were negative and faint in DCT. We found that camel kidneys showed that WGA and SBA were moderate and mild respectively while UEA and RCA were mild and moderate respectively in DCT. Our study revealed that lectins expression in PCT and DCT is lower in camel than in cow.

Yabuki *et al.* (2012) showed that in dog, UEA-I was specific to the distal tubules (thick ascending limb and DCT) and no signals in bowman capsule and glomeruli. This positive cells of UEA localization was similar to previous study which compared between 14 different animal species including the dog (Holthofer, 1983) and on the other hand, we found that UEA signals in cat were moderate in bowman capsule and glomeruli, PCT and DCT. Our study revealed that UEA in cat were also higher than dog.

Yabuki *et al.* (2012) showed that in dog, RCA-I stained heterogeneously in glomeruli, capillary endothelial cells, no reaction in bowman capsule, strong intensity in PCTs and PCTs which in contrast with our data in cat as we found that RCA stained homogeneously in both kidneys showed mild reaction

in glomerular capillaries, bowman's capsule while moderate in podocyte, intense reaction in DCT and PCT. We found that SBA showed intense signals in bowman capsule, moderate in podocytes, PCT and mild in DCT and on the other hand, Yabuki *et al.* (2012) showed that in dog, SBA showed intense staining in thin limb of loop of Henle and DCT and no in bowman capsule or glomeruli while in our data in cat.

Finally, our study revealed that different lectin localizations between camel and cat. Concerning bowman capsule, WGA were intense in camel and other four lectins are moderate. While in cat kidney, signals of SBA, UEA and RCA were intense, moderate and mild respectively. In glomeruli, there were no lectins signals in camel while Con A and RCA were intense and mild in cat. In PCT, there were no lectin signals in camel except SBA which was moderate while RCA was intense in cat and other signals were moderate. In DCT, all lectins signals were moderate in camel while RCA, Con A were intense and other lectins were moderate. Therefore, most of lectins were concentrated in bowman capsule in camels while in cat, lectins localized mainly in DCT and PCT and glomeruli. The camel showed higher lectins in bowman capsule and this may relate to glomerular filtration and water conservation of camel.

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