

## ORIGINAL ARTICLE

# Age-related changes in urinary protein excretion in relation to indices of renal function in Wistar rats

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**Abstract**

**Background:** The study determined the fractions of proteins in the urine and plasma of rats at different ages, measured the plasma and urine concentrations of markers of renal function, with a view to determining the influence of proteinuria on renal function.

**Methods:** Eighty Wistar rats were used for this study. Groups 1 and 2 each consisted of eight 1-month-old male and female rats; 3 and 4 had eight 3-month-old male and female rats; 5 and 6 had eight 6-month-old male and female rats; 7 and 8 had eight 9-month old male and female rats; and 9 and 10 had eight 12-month-old male and female rats.

**Results:** A fraction of the molecular weight of protein in the urine of rats aged 1, 9 and 12 months was higher than that of 3 and 6 months. The total protein concentration in the urine of male and female rats aged 9 and 12 months was significantly higher than that of rats aged 1 and 3 months. The urine creatinine concentrations of male and female rats aged 9 months were significantly higher when compared with that of 1, 3, 6 and 12 months.

**Conclusion:** Our results suggest that the 3-month-old rats seem less affected by proteinuria, because they had the least urine protein, and consistent and reduced plasma and urine concentrations of markers of renal function. The results of this study may provide a foundation for future mechanistic inquiries as to why this age group was the least affected by proteinuria.

**KEYWORDS**

creatinine, electrophoresis, protein, rats, urea

## 1 | INTRODUCTION

Proteinuria is the presence of excess serum proteins in the urine. In humans, abnormal passage of plasma proteins across the glomerular capillary wall to the urinary space is the common event in many nephropathies in which glomerular membrane permeability is altered by the underlying disease.<sup>1</sup>

Wistar rats, often used for comparative investigations of renal physiology and pathology, are known to excrete a considerable amount of protein in their urine under normal conditions. There is evidence that proteins are produced in the liver and excreted in the urine.<sup>2</sup> Proteinuria begins at approximately 2 months of age in the male rat and increases progressively through senescence, or approximately 2 years of age.<sup>3-5</sup> The presence of protein in the urine of

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healthy rats could interfere with experimentally-induced conditions such as diabetes, hypertension and obesity, thereby complicating the interpretation of results.<sup>6</sup>

Previous studies on proteinuria in rats did not demonstrate how changes in urine protein with age affect the plasma and urine concentrations of other markers of renal function. Findings from studies on renal physiology and pathology using experimental animals are intended to be applied to human health and life. In such cases, using rats of random age or weight rather than precisely correlated age could limit the accuracy of the experiment. This study is therefore aimed at relating the changes in urine protein excretion in rats at different ages with plasma and urine concentrations of some selected markers of renal function.

## 2 | MATERIALS AND METHODS

### 2.1 | Animal management

Eighty Wistar rats of both sexes at 3 weeks of age purchased from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, were used for this study. The rats were kept under natural light/dark cycle and allowed free access to standard rodent pellet and water ad libitum.

### 2.2 | Ethics statement

All experimental protocols were in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Science<sup>7</sup> and approved by the Health Research and Ethics Committee of Public Health of Obafemi Awolowo University (reference no. IPHOAU/12/919).

### 2.3 | Experimental design

Ten groups of eight rats each were studied. Groups 1 and 2 each consisted of eight juveniles (1 months old) male and female rats; 3 and 4 had eight young adult (3 months old) male and female rats; 5 and 6 had eight adults (6 months old) male and female rats; 7 and 8 had eight aged (9 months old) male and female rats; and 9 and 10 had eight aged (12 months old) male and female rats. At the end of the study period, urine samples of the rats were collected over a 24-hours period using metabolic cages. Thereafter, they were anaesthetized with ketamine hydrochloride (10 mg/kg; Rotex Medica, Trittau, Germany) via i.m. route. Blood samples from each rat were collected by cardiac puncture into separate fluoride oxalate bottles. The samples were then centrifuged at 1700 g for 15 minutes at  $-4^{\circ}\text{C}$  using a cold centrifuge (Model 8881; Centurion Scientific, Centurion Scientific Limited, Stoughton, UK) to separate out the plasma.

### 2.4 | Biochemical assay

Total protein was determined according to the method of Lowry et al.<sup>8</sup> Creatinine, urea, albumin and glucose concentrations in

the plasma and urine were assayed using appropriate biochemical kits purchased from Randox Laboratories (Crumlin, Antrim, UK).

Creatinine clearance was calculated using the standard formula as follows:

$$\text{Creatinine clearance} = \text{UcV/Pc (mL/min)}$$

where Uc = concentration of creatinine in urine, V = urine flow rate = amount of urine/time (min) and Pc = concentration of creatinine in plasma.

### 2.5 | Polyacrylamide gel electrophoresis

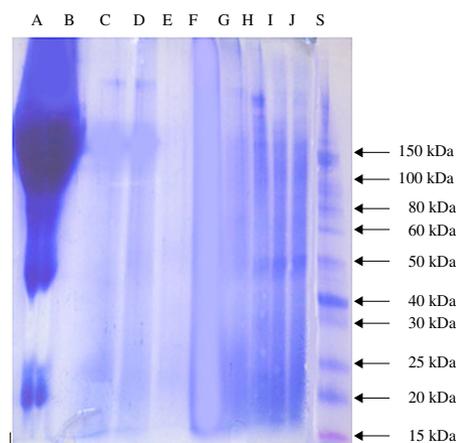
The subunits of the molecular weights of proteins in the plasma and urine of the rats were determined by polyacrylamide gel electrophoresis according to the method of Laemmli.<sup>9</sup>

### 2.6 | Histological processes

The kidneys of the rats were fixed in 10% formol-saline. Thereafter, they were dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax. The tissues were then cut into 3–4- $\mu\text{m}$  thick sections by a microtome, fixed on the slides and stained with hematoxylin-eosin. The slides were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) and photomicrographs were taken with a Leica DM 750 camera at  $\times 400$  magnification (Leica Microsystems, Wetzlar, Germany).

### 2.7 | Statistical analysis

The results obtained were expressed as mean  $\pm$  standard error of the mean. Statistical analysis comparing the age-related differences among the five groups were analyzed using one-way ANOVA followed by Newman-Keuls multiple comparison test using GraphPad version 5.03 software (GraphPad Software, La Jolla, CA, USA). Student's



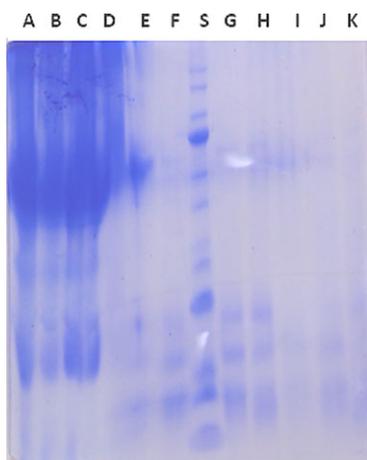
**FIGURE 1** Polyacrylamide gel electrophoresis of plasma and urine of male and female rats at 1 month of age. Standard protein marker of known molecular weights are shown on lane S. Electrophoretograms of the plasma of male and female rats are denoted in lane A and B, respectively, while those of their urine are shown in lanes C, D–F and G–J, respectively

*t* test was used for paired observation. The results were considered significant when  $P < 0.05$ .

### 3 | RESULTS

#### 3.1 | Proteins in the plasma of Wistar rats

The plasma from Wistar rats of different ages were subjected to electrophoretic analysis and varying protein patterns were obtained. Figures 1-3 revealed the presence of low molecular weight proteins of between 7.83 and 33.99 kDa in the plasma of 1-, 3- and 6-month-old male and female rats, although there was presence of high molecular weight protein representing a small fraction of the total proteins in their plasma (Figures 1-3).



**FIGURE 2** Polyacrylamide gel electrophoresis of plasma and urine of 3-month-old rats of either sex. Lane S shows standard protein marker of known molecular weights. Electrophoretograms of the plasma of rats of both sexes are shown in lanes A, B and C, D, respectively. Lanes E-H and I-K describe the electrophoretograms of the urine of male and female rats respectively

However, there was a marginal discrepancy in the protein patterns obtained from 9- and 12-month-old male and female rats. The electrophoresis revealed the presence of both low and high molecular weight proteins in the plasma of 9- and 12-month-old male rats of between 13.43 and 24.24 kDa and 58.21 and 244.3 kDa, respectively. The protein patterns of their female counterparts consisted mainly of low molecular weight proteins of between 10.13 and 17.55 kDa but high molecular weight protein represented a small fraction of the total protein of between 60.70 and 94.90 kDa (Figures 4-6).

#### 3.2 | Protein excretion in the urine of Wistar rats

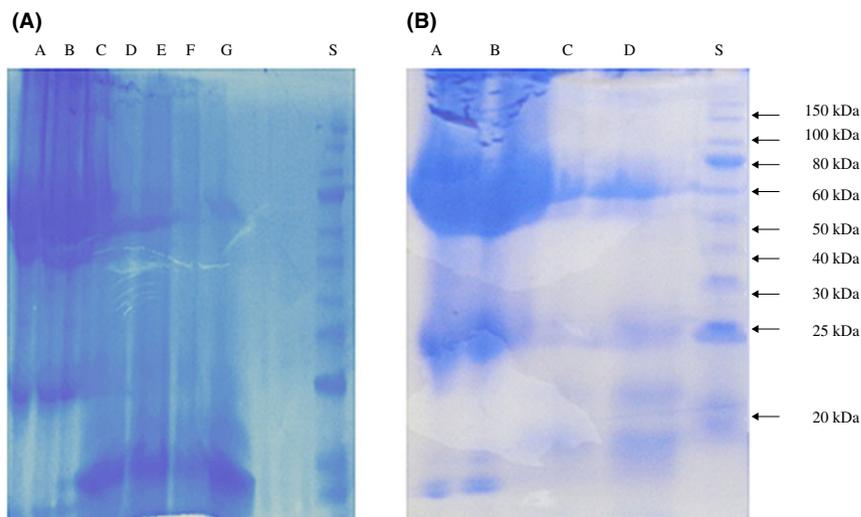
For analysis of the urinary proteins, the urine of Wistar rats of different ages were also subjected to electrophoresis. High molecular weight proteins of between 126.46 and 180.15 kDa were found in the urine of 1-month-old male and female rats. However, a small fraction of low molecular weight protein of 43.77 kDa was seen in the females (Figure 1). In contrast, low molecular weight proteins of between 12.48 and 23.80 kDa and 14.94 and 27.09 kDa, respectively, were found in the urine of 3- and 6-month-old male and female rats. However, a high molecular weight protein of 84.50 kDa and 86.35 kDa, respectively, represented a small fraction of the total protein excretion in the males (Figures 2 and 3).

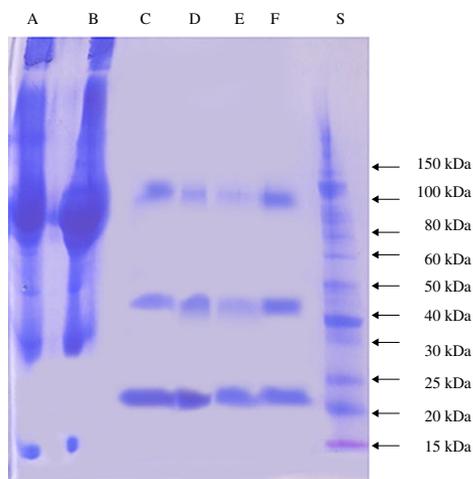
The results obtained from 9- and 12-month-old male and female rats were similar to those of 3- and 6-month-old rats. Low molecular weight proteins of between 19.29 and 10.13 kDa, 28.23 and 26.18 kDa, respectively, were observed, although a small protein fraction of high molecular weight of 131.16 and 84.04 kDa and 134.59 and 107.08 kDa, respectively, were also present (Figures 4-6).

#### 3.3 | Age-related differences in plasma concentrations of some markers of renal function in male Wistar rats

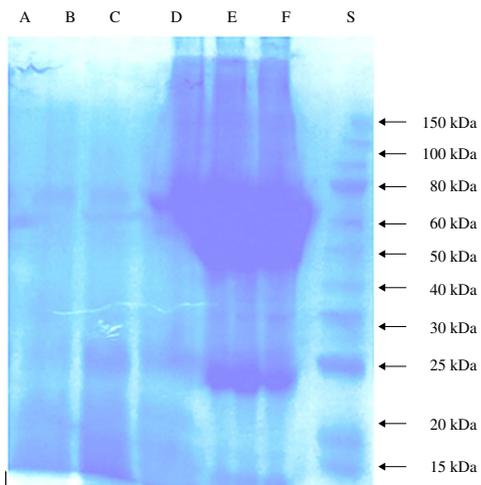
The plasma creatinine concentration was significantly lower in 1- and 3-month-old male rats when compared with rats in other age

**FIGURE 3** Polyacrylamide gel electrophoresis of plasma and urine of male and female rats at 6 months of age. Standard protein marker of known molecular weights are shown in lane S. Electrophoretic pattern of the plasma of male (left) and female (right) rats are shown in lane A-C and A, B, respectively. Electrophoretic patterns of their urine are depicted in lanes D-G and C, D, respectively





**FIGURE 4** Polyacrylamide gel electrophoresis of plasma and urine of male rats aged 9 months. Standard protein marker of known molecular weights are shown in lane S. Electrophoretograms of the plasma of male rats are shown in lane A and B while those of their urine are represented in lanes C-F

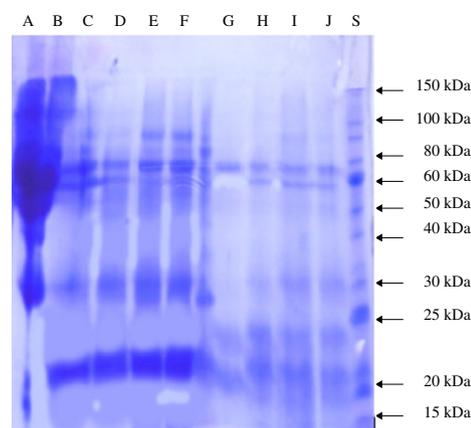


**FIGURE 5** Polyacrylamide gel electrophoresis of plasma and urine of female rats aged 9 months. Standard protein marker of known molecular weights is shown in lane S. Electrophoretic bands of the urine of female rats are shown in lanes A-C. Lanes D-F represent the electrophoretic bands of their plasma

groups with the 3-month-old rats having the lowest plasma creatinine ( $F = 6.037$ ,  $P = 0.002$ ).

A significantly lower total protein concentration was also recorded in the male rats aged 1 and 3 months when compared with rats of aged 6, 9 and 12 months ( $F = 398.4$ ;  $P = 0.0001$ ) with the 3-month-old rats having the least total protein.

The albumin concentration of 1-month-old rats was significantly higher ( $F = 191.8$ ,  $P = 0.0001$ ) than that of 3-, 6- and 9-month-old rats but significantly lower when compared with 12-month-old rats. Also, rats aged 3 months had a plasma albumin concentration that was significantly higher ( $t = 9.301$ ,  $P = 0.004$ ) than that of 6-month-old rats but significantly lower ( $t = 15.71$ ,  $P = 0.0001$ ) when compared with 12-month-old rats. Rats at 6 months of age had the



**FIGURE 6** Polyacrylamide gel electrophoresis of plasma and urine of rats of both sexes at 12 months of age. Lane S shows standard protein marker of known molecular weights. Electrophoretograms of the plasma of male and female rats are shown in lane A and B, respectively while those of their urine are depicted in lanes C-F and G-J, respectively

lowest plasma albumin concentration when compared with that of other age groups ( $F = 231.1$ ,  $P = 0.0001$ ).

Plasma glucose concentrations of 6-, 9- and 12-month-old rats were significantly lower ( $F = 26.59$ ,  $P = 0.0001$ ) than those of rats aged 1 and 3 months. Also, rats aged 6 months had a plasma glucose concentration that was significantly lower ( $F = 15.19$ ,  $P = 0.0002$ ) when compared with that of 9 and 12 months. Rats aged 9 months had a plasma urea concentration that was significantly higher ( $F = 28.98$ ,  $P = 0.0001$ ) than that of other age groups (Table 1).

### 3.4 | Age-related differences in urine concentrations of some markers of renal function in male Wistar rats

The concentration of creatinine in the urine of 6- and 9-month-old rats was significantly higher than that in rats aged 1, 3 and 12 months ( $F = 11.46$ ,  $P = 0.0001$ ) and the 9-month-old rats had the highest urine creatinine concentration.

Total protein concentrations in the urine of rats aged 1 and 3 months were significantly lower ( $F = 23.88$ ,  $P = 0.0001$ ) when compared with those of rats aged 9 and 12 months. Also, the urine total protein concentration of rats aged 3 months was significantly lower ( $t = 4.219$ ,  $P = 0.002$ ) than that of rats aged 6 months. The urine albumin concentrations of 1- and 12-month-old rats were significantly higher ( $F = 163.5$ ,  $P = 0.0001$ ) than those of 3-, 6- and 9-month-old rats.

Also, rats aged 1, 3 and 6 months had urine glucose concentrations that were significantly lower ( $F = 4.906$ ,  $P = 0.004$ ) when compared with rats aged 9 and 12 months.

Plasma urea increased significantly in the 9-month-old male rats when compared with rats of other age groups ( $F = 32.27$ ,  $P = 0.0001$ ). The urine concentration of urea in rats aged 1 month

**TABLE 1** Age-related differences in plasma concentration and urinary excretion of some markers of renal function in male Wistar rats

	Age (months)				
	1	3	6	9	12
<b>Plasma</b>					
Creatinine (mg/dL)	0.59 ± 0.11	0.22 ± 0.01 <sup>a</sup>	0.85 ± 0.05 <sup>b</sup>	1.19 ± 0.03 <sup>a,b</sup>	1.91 ± 0.07 <sup>a,b</sup>
Total protein (g/dL)	8.18 ± 0.14	2.92 ± 0.28 <sup>a</sup>	16.39 ± 0.49 <sup>a,b</sup>	17.49 ± 0.37 <sup>a,b</sup>	10.85 ± 0.12 <sup>a,b,c,d</sup>
Albumin (g/dL)	6.66 ± 0.19	3.49 ± 0.03 <sup>a</sup>	2.33 ± 0.13 <sup>a,b</sup>	3.37 ± 0.10 <sup>a,c</sup>	7.79 ± 0.25 <sup>a,b,c,d</sup>
Glucose (mmol/L)	7.44 ± 0.34	7.93 ± 0.45	4.00 ± 0.09 <sup>a,b</sup>	5.28 ± 0.21 <sup>a,b,c</sup>	4.97 ± 0.19 <sup>a,b</sup>
Urea (mmol/L)	8.84 ± 0.69	10.23 ± 0.53	8.39 ± 0.29	20.41 ± 2.18 <sup>a,b,c</sup>	6.41 ± 0.38 <sup>d</sup>
<b>Urine</b>					
Creatinine (mg/dL)	0.22 ± 0.06	0.36 ± 0.09	1.68 ± 0.49 <sup>a,b</sup>	9.08 ± 2.48 <sup>a,b,c</sup>	0.25 ± 0.09 <sup>c,d</sup>
Total protein (g/dL)	4.06 ± 0.28	3.59 ± 0.36	5.49 ± 0.26 <sup>b</sup>	8.08 ± 0.42 <sup>a,b</sup>	7.33 ± 0.48 <sup>a,b</sup>
Albumin (g/dL)	4.46 ± 0.35	0.55 ± 0.03 <sup>a</sup>	0.41 ± 0.01 <sup>a</sup>	0.69 ± 0.06 <sup>a</sup>	5.23 ± 0.24 <sup>b,c,d</sup>
Glucose (mmol/L)	0.70 ± 0.09	0.69 ± 0.03	0.66 ± 0.07	1.01 ± 0.09 <sup>a,b,c</sup>	1.09 ± 0.14 <sup>a,b,c</sup>
Urea (mmol/L)	16.74 ± 0.99	34.20 ± 3.46	62.40 ± 1.62	183.3 ± 26.89 <sup>a,b,c</sup>	22.47 ± 0.17 <sup>c,d</sup>

Values are expressed as mean ± standard error of the mean (n = 8).  $P < 0.05$ .

<sup>a</sup>Significantly different from 1-month-old rats.

<sup>b</sup>Significantly different from 3-month-old rats.

<sup>c</sup>Significantly different from 6-month-old rats.

<sup>d</sup>Significantly different from 9-month-old rats.

was significantly lower ( $F = 102.1$ ,  $P = 0.0001$ ) when compared with 3- and 6-month-old rats (Table 1).

### 3.5 | Age-related differences in plasma concentrations of some markers of renal function in female Wistar rats

The plasma creatinine concentration of 3-month-old rats was lower than that of other age groups but it was only significant ( $F = 2.675$ ,  $P = 0.015$ ) when compared with 12-month-old rats.

The concentrations of total protein in the plasma of 1-, 9- and 12-month-old female rats were significantly higher ( $F = 655.9$ ,  $P = 0.0001$ ) when compared with 3- and 6-month-old rats. However, the plasma total protein of 1-month-old rats was significantly lower ( $F = 154.4$ ,  $P = 0.0001$ ) than that of rats aged 9 and 12 months.

The plasma albumin concentrations were significantly lower in rats aged 3, 6 and 9 months when compared with rats aged 1 and 12 months ( $F = 49.18$ ,  $P = 0.0001$ ). In addition, the plasma albumin concentration of female rats aged 1 month was significantly lower ( $t = 3.383$ ,  $P = 0.0061$ ) than that of the rats aged 12 months.

The plasma glucose concentrations of 6-, 9- and 12-month-old rats were significantly lower ( $F = 11.62$ ,  $P = 0.0001$ ) than those of 1- and 3-month-old rats. The plasma urea concentration of 3-month-old rats was significantly higher ( $F = 9.867$ ,  $P = 0.0001$ ) when compared with rats aged 1, 6, 9 and 12 months (Table 2).

### 3.6 | Age-related differences in urine concentrations of some markers of renal function in female Wistar rats

Female rats aged 9 months had a urine creatinine concentration that was significantly higher ( $F = 17.56$ ,  $P = 0.0001$ ) than that of other

age groups, while that of rats aged 1 and 3 months was significantly lower ( $F = 17.56$ ,  $P = 0.0001$ ) when compared with 6- and 12-month-old rats.

The urine total protein concentrations were observed to be significantly lower in female rats at 1 and 3 months of age when compared with rats aged 6, 9 and 12 months ( $F = 16.47$ ,  $P = 0.0001$ ). Also, a significantly lower urine total protein was recorded in the female rats at 6 months of age when compared with rats at 9 months of age ( $t = 4.593$ ,  $P = 0.001$ ). The urine albumin concentration of rats at 1 and 12 months of age was significantly higher ( $F = 2039$ ,  $P = 0.0001$ ) than that of rats aged 3, 6 and 9 months.

The glucose concentration in the urine of 6-month-old rats was significantly higher ( $F = 3.522$ ,  $P = 0.020$ ) when compared with 1- and 3-month-old rats.

The urea concentration in the urine of 1-month-old rats was significantly lower ( $F = 22.55$ ,  $P = 0.0001$ ) when compared with rats aged 3, 6, 9 and 12 months. The concentration of urea in the urine of rats aged 3 and 6 months was significantly higher ( $F = 11.33$ ,  $P = 0.0001$ ) than that of 9- and 12-month-old rats (Table 2).

### 3.7 | Age-related differences in creatinine clearance and urine volume of male Wistar rat

Significantly higher renal creatinine clearances were recorded in the male rats aged 3, 6 and 9 months when compared with 1- and 12-month-old rats ( $F = 3.195$ ,  $P = 0.029$ ). The urine volume of 1-, 9- and 12-month-old rats was lower but not significantly different ( $F = 2.178$ ,  $P = 0.097$ ) when compared with 3- and 6-month-old rats.

The creatinine clearance of the female rats at 1 month of age was significantly lower than that of other age groups ( $F = 3.258$ ,  $P = 0.030$ ). Also, the clearances of 3- and 9-month-old rats were

**TABLE 2** Age-related differences in plasma concentration and urinary excretion of some markers of renal function in female Wistar rats

	Age (months)				
	1	3	6	9	12
Plasma					
Creatinine (mg/dL)	1.23 ± 0.68	0.14 ± 0.02 <sup>a</sup>	0.89 ± 0.03	1.09 ± 0.14	1.87 ± 0.52 <sup>b</sup>
Total protein (g/dL)	8.04 ± 0.12	3.06 ± 0.18 <sup>a</sup>	2.95 ± 0.21 <sup>a</sup>	14.00 ± 0.18 <sup>a,b,c</sup>	16.30 ± 0.38 <sup>a,b,c,d</sup>
Albumin (g/dL)	5.92 ± 0.18	3.65 ± 0.10 <sup>a</sup>	2.98 ± 0.16 <sup>a</sup>	3.49 ± 0.12 <sup>a</sup>	8.26 ± 0.62 <sup>a,b,c,d</sup>
Glucose (mmol/L)	6.36 ± 0.51	7.54 ± 0.59	4.38 ± 0.42 <sup>a,b</sup>	4.71 ± 0.19 <sup>a,b</sup>	4.09 ± 0.28 <sup>a,b</sup>
Urea (mmol/L)	6.15 ± 0.46	11.52 ± 1.18 <sup>a</sup>	4.27 ± 0.52 <sup>b</sup>	5.34 ± 0.33 <sup>b</sup>	5.74 ± 1.30 <sup>b</sup>
Urine					
Creatinine (mg/dL)	0.26 ± 0.13	0.77 ± 0.09	3.26 ± 0.37 <sup>a,b</sup>	8.03 ± 1.45 <sup>a,b,c</sup>	3.05 ± 0.17 <sup>a,b,c,d</sup>
Total protein (g/dL)	3.09 ± 0.15	3.34 ± 0.39	5.19 ± 0.23 <sup>a,b</sup>	7.09 ± 0.33 <sup>a,b,c</sup>	5.70 ± 0.62 <sup>a,b,d</sup>
Albumin (g/dL)	5.18 ± 0.07	0.61 ± 0.03 <sup>a</sup>	0.63 ± 0.01 <sup>a</sup>	0.74 ± 0.07 <sup>a</sup>	5.73 ± 0.08 <sup>b,c,d</sup>
Glucose (mmol/L)	0.63 ± 0.04	0.68 ± 0.02	1.05 ± 0.16 <sup>a,b</sup>	0.85 ± 0.09	0.85 ± 0.05
Urea (mmol/L)	14.03 ± 1.48	30.51 ± 2.02 <sup>a</sup>	28.80 ± 1.09 <sup>a</sup>	22.96 ± 0.89 <sup>a,b,c</sup>	22.22 ± 0.26 <sup>a,b,c</sup>

Values are expressed as mean ± standard error of the mean (n = 8). *P* < 0.05.

<sup>a</sup>Significantly different from 1-month-old rats.

<sup>b</sup>Significantly different from 3-month-old rats.

<sup>c</sup>Significantly different from 6-month-old rats.

<sup>d</sup>Significantly different from 9-month-old rats.

significantly higher when compared with 6- and 12-month-old rats (*F* = 3.258, *P* = 0.030) (Table 3).

### 3.8 | Photomicrographs of the kidney cortex of male and female Wistar rats

The photomicrographs of the male and female kidney cortex show normal glomeruli. Normal Bowman's spaces were noted. The proximal convoluted tubule and distal convoluted tubule appear normal across the age groups (hematoxylin-eosin) (Figures 7 and 8).

## 4 | DISCUSSION

Electrophoresis has been a useful method in proteomics for protein analysis. We therefore employed this technique as a comparative

procedure for plasma and urinary proteins present in Wistar rats with respect to their age and sex.

The type of protein excreted is of importance for correlating structure and function of the kidney, because excretion of protein differs with sex and age.<sup>10,11</sup> Electrophoretic patterns of proteins obtained in the urine of 1- and 12-month-old rats revealed they were of high molecular weights, while those obtained from 3- and 6-month-old rats were low molecular weight proteins.

Low molecular weight proteins are produced in the liver and under the control of hormones such as androgens.<sup>2</sup> Excretion of low molecular weight protein is not indicative of underlying renal pathology. Albumin, on the other hand, is a high molecular weight plasma protein which escapes into the urine when the permeability of the glomerular filtration barrier has been altered.<sup>12,13</sup> High molecular weight proteins begin to appear in the urine of 1-month-old rats of both sexes and decrease thereafter in the 3- and 6-month-old rats,

**TABLE 3** Age-related differences in Ccr and urine volume in male and female Wistar rats

	Age (months)				
	1	3	6	9	12
Male					
Ccr (×10 <sup>-3</sup> mL/min)	0.53 ± 0.0002	2.99 ± 0.001 <sup>a</sup>	3.17 ± 0.001 <sup>a</sup>	7.43 ± 0.002 <sup>a</sup>	0.27 ± 0.0002 <sup>b,c,d</sup>
Urine volume (mL)	1.63 ± 0.29	2.17 ± 0.31	2.33 ± 0.33	1.52 ± 0.35	1.35 ± 0.25 <sup>b,c</sup>
Female					
Ccr (×10 <sup>-3</sup> mL/min)	2.21 ± 0.002	5.06 ± 0.001 <sup>a</sup>	3.79 ± 0.001 <sup>a,b,d</sup>	11.56 ± 0.004 <sup>a</sup>	4.24 ± 0.001 <sup>a,b,d</sup>
Urine volume (mL)	1.28 ± 0.14	1.12 ± 0.08	1.40 ± 0.21	1.89 ± 0.29	2.87 ± 0.26 <sup>a,b,c</sup>

CCR, creatinine clearance.

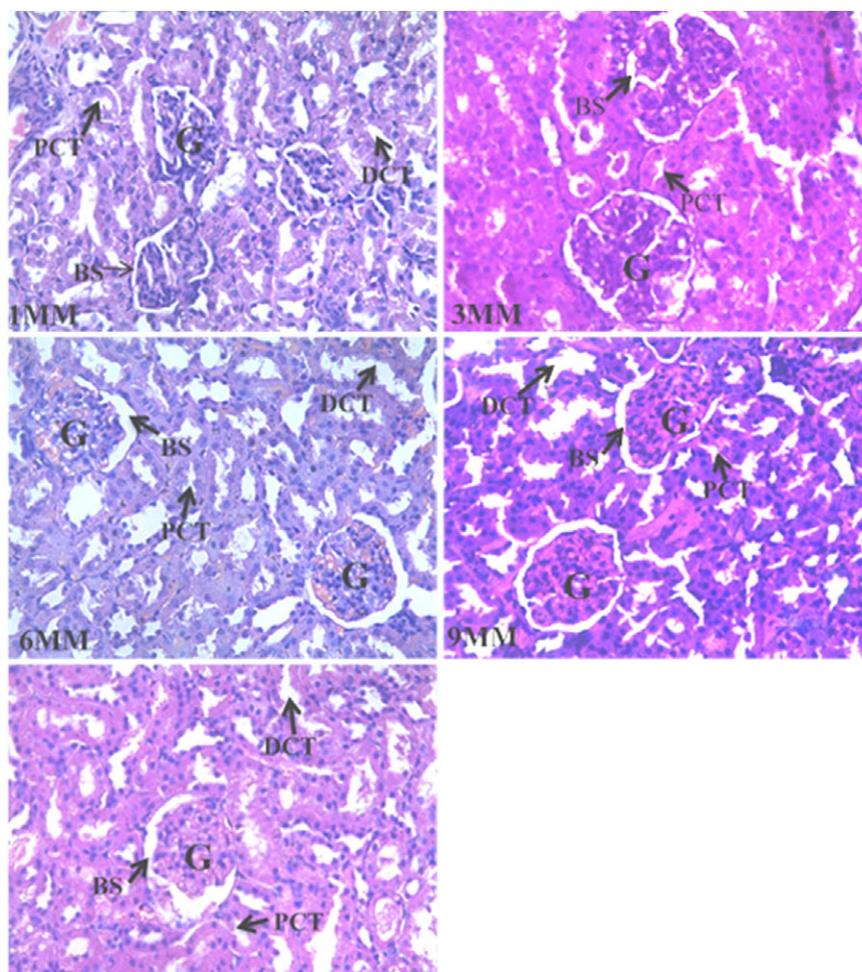
Values are expressed as mean ± standard error of the mean (n = 8).

<sup>a</sup>Significantly different from 1-month-old rats.

<sup>b</sup>Significantly different from 3-month-old rats.

<sup>c</sup>Significantly different from 6-month-old rats.

<sup>d</sup>Significantly different from 9-month-old rats.



**FIGURE 7** Photomicrographs of the male kidney cortex (1MM, 1-month-old male rats; 3MM, 3-month-old male rats; 6MM, 6-month-old male rats; 9MM, 9-month-old male rats; 12MM, 12-month-old male rats) showing normal glomeruli (G). Normal Bowman's spaces (BS) were noted. The proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) appear normal across the age groups (hematoxylin–eosin, original magnification  $\times 400$ )

after which they later increase in the 9- and 12-month-old rats. It is therefore of considerable significance that functional abnormalities in the renal corpuscle have been noted as early as 1 month of age in rats of both sexes, although pathological changes are much more common at older age.<sup>14–17</sup> This suggested more strongly than not that the observed change in urinary protein excretion was primarily the result of a change in the rate of protein filtration at the glomerulus rather than reabsorption of protein within the tubules.<sup>18</sup>

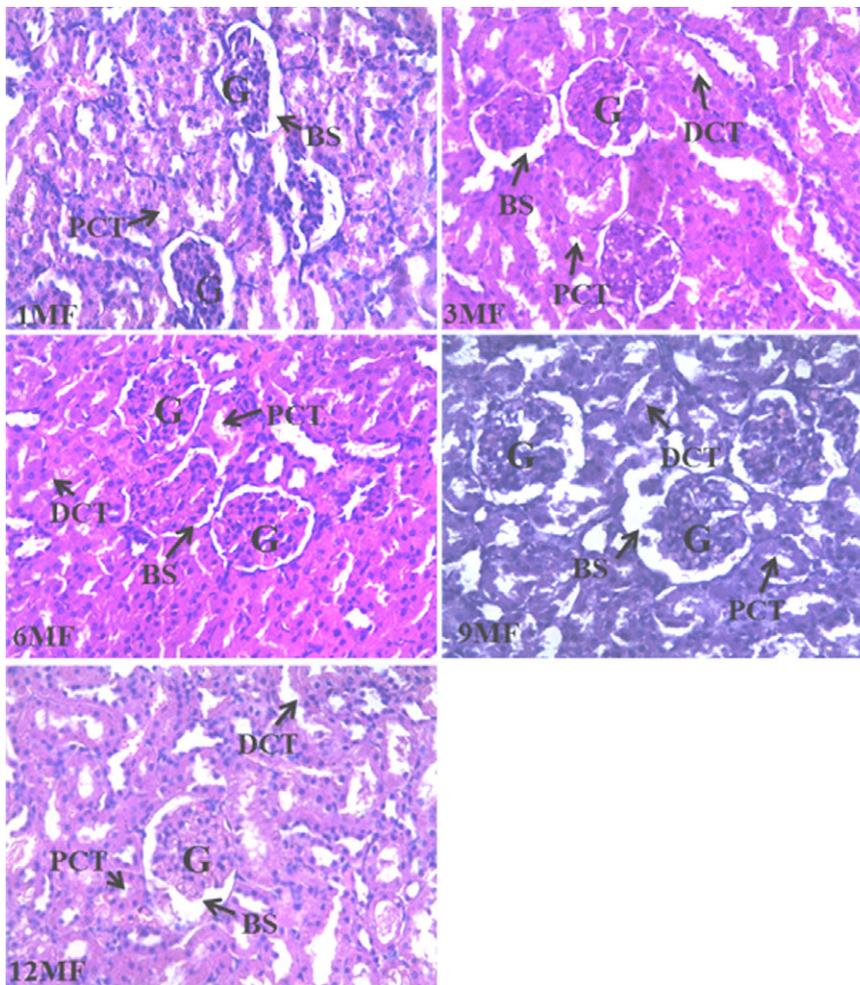
The fact that proteinuria was already evident in 1-month-old rats indicates an early onset of glomerular dysfunction, even before extensive changes are observed histologically. Thus, it seems that the selective determination of protein excretion by electrophoresis will be a good indicator in glomerular permeability studies. The presence of protein in the urine of 1-month-old rats showed that proteinuria begins at approximately 1 month of age in Wistar rats.

The total protein concentrations in the urine of male and female rats aged 9 and 12 months were significantly higher when compared with those of rats aged 1 and 3 months. Age-related renal functional changes and lesions in rats have been reported by different researchers.<sup>19–22</sup> Aging is associated with proteinuria, glomerulosclerosis and progressive renal impairment.<sup>23,24</sup> The photomicrographs of the male and female kidney cortex show normal glomeruli. Normal Bowman's spaces were also noted. The proximal convoluted tubule

and distal convoluted tubule appear normal across the age groups (Figures 1 and 2). This indicated that such proteinuria may occur in the absence of any underlying pathological process. Rather, it appears to be the consequence of adaptive changes which occur in residual nephrons when the total nephron population is reduced.<sup>25</sup> It is therefore assumed that an alteration in glomerular permselectivity is an integral part of the adaptive changes which occur when nephron mass is reduced and that it is this change which is primarily responsible for the increased urinary protein excretion observed in non-glomerular renal diseases.

Rats of both sexes at 12 months of age had the highest level of plasma creatinine when compared with rats of other age groups. This might have resulted from reduction in glomerular filtration rate, because creatinine concentration in the plasma depends largely on glomerular function<sup>26</sup> or storage of creatinine as a waste product in muscles. The decrease in plasma creatinine in the 3-month-old rats of both sexes may be an expression of improved filtration rate or diminished muscle metabolism.

It is well known that creatinine is a breakdown product of creatine phosphate in muscle.<sup>27</sup> A significantly higher urine creatinine concentration was observed in 9-month-old rats of either sex when compared with other age groups. The urine creatinine concentration of the rats increased gradually and peaked at the age of 9 months



**FIGURE 8** Photomicrographs of the female kidney cortex (1MF, 1-month-old female rats; 3MF, 3-month-old male rats; 6MF, 6-month-old female rats; 9MF, 9-month-old female rats; 12MF, 12-month-old female rats) showing normal-appearing glomeruli (G). Normal Bowman's spaces (BS) were prominent. The proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) appear intact in all the age groups (hematoxylin–eosin, original magnification  $\times 400$ )

with a subsequent decline at 12 months. The urine concentration of excretory product depends almost entirely on tubular function.<sup>28</sup> Hence, the reduced urine creatinine concentration was evidence of decreased ability of the renal tubules to remove and excrete a large quantity of creatinine into the urine of the rats. Plasma urea increased significantly in the 9-month-old male rats when compared with rats of other age groups. This may be due to increased urea reabsorption at the collecting duct resulting from low urine flow rates and to the diffusion of urea from the terminal collecting ducts into the medullary interstitium. A portion of this urea fails to be recycled within the kidney and thus returns to the general circulation.<sup>29</sup>

The ability to control precisely the blood glucose level in humans and experimental animals declines with age. Rats have been reported to show a decrease in peripheral glucose uptake<sup>30</sup> as they age. The plasma glucose concentrations of 6-, 9- and 12-month-old rats of both sexes were significantly lower than those of 1- and 3-month-old rats. This finding is in contrast with the report of Ghezzi et al,<sup>31</sup> who reported that Wistar rats at 12 months of age showed no significant difference in blood glucose level when compared with rats at 2, 4 and 6 months of age despite the increase in bodyweight and adipose hyperplasia.<sup>32</sup> The observed discrepancy between the

present study and that of Ghezzi et al<sup>31</sup> might have resulted from differences in age between rats used in each study. This study used rats of 1, 3, 6, 9 and 12 months of age while their study used rats of 2, 4, 6 and 12 months of age. The decrease in plasma glucose concentration that was found in the older rats when compared with the young rats may be due maintenance of high-circulating triglyceride concentration caused by an imbalance in lipid and carbohydrate oxidation.<sup>33</sup>

Normally, approximately 60%–65% of tubular reabsorption occurs in the proximal convoluted tubules with close to 100% of glucose reabsorbed.<sup>34</sup> With aging, rat tubules have been shown to have non-uniform thickening of the tubular basement membrane with vacuoles in the proximal tubules and intermittent loss of the microvilli.<sup>17</sup> The urine glucose concentrations of the 9- and 12-month-old rats was higher than those of other age groups. Thus, the increase in their urine glucose could be attributed to impaired proximal tubular function, where the majority of glucose reabsorption takes place.

#### 4.1 | Urine volume

The volume of urine voided depends on the degree of hydration, glomerular filtration and tubular function. Renal diuresis, which did

not appear to be related to endocrine dysfunction, has previously been reported in aging male Sprague-Dawley rats.<sup>35</sup> This study showed that passage of a large volume of urine also occurs in female rats. In the present study, the urine volume of the male rats at 1, 9 and 12 months of age was lower than that of 3- and 6-month-old rats. However, the urine volume of the female rats at 12 months of age was significantly higher when compared with 1-, 3- and 6-month-old rats. The higher volume of urine excreted by female Wistar rats may be due to decrease ability of their kidneys to concentrate urine.<sup>36,37</sup> The impairment of the urine concentrating ability of the kidney may have resulted from a defect in the concentrating gradient in the medullary region with loss of the osmotic gradient between the plasma and urine.<sup>38,39</sup>

A study of the medulla of aged rats also suggested a decrease in many key transport proteins that participate in urine concentrating ability (aquaporins, urea transporters, V<sub>2</sub> receptor) with reduced response to water restriction.<sup>38</sup> This could also explain the increased urine volume that was observed in the female rats at 12 months of age.

## 4.2 | Creatinine clearance

Senescence is associated with a gradual reduction in the glomerular filtration rate. Previous reports on renal aging in rats<sup>40,41</sup> demonstrated gradual reduction in creatinine clearance up to 12 months of age, corroborating the results of this study which showed significant decrease in the renal creatinine clearance in male rats aged 1 and 12 months when compared with 3-, 6- and 9-month-old rats. Renal blood flow of aged men has been found to decline by 10%/year after the age of 40 years.<sup>42,43</sup> Thus, the decreased creatinine clearance in rats of this age group might have resulted from reduced renal blood flow. The decrease in renal blood flow may result from an imbalance and alterations in the responsiveness to vasoactive substances, namely acetylcholine, histamine and prostaglandins,<sup>44</sup> or decrease in production of certain peptides associated with aging.<sup>43,45</sup>

## 4.3 | Conclusion

From the findings of this study, it appears that 3-month-old rats seem less affected by proteinuria. This was based on the premise that they excreted low molecular weight protein in their urine, with reduced and consistent plasma and urine concentrations of markers of renal function that were assessed when compared with rats of other age groups. However, the specificity and sensitivity of the carrier protein transporter required for movement of substances from the tubular epithelial cells into renal interstitium across the different age groups were not examined in this study. The results of this study may provide a foundation for future mechanistic inquiries on the influence of proteinuria on markers of renal function in rats of different age group.

## CONFLICTS OF INTEREST

None.

## AUTHOR CONTRIBUTIONS

OSO was involved in the conception and design of the work, acquisition and interpretation of data. He also participated in drafting the article and revising it critically for intellectual content. ARO initiated the research idea. He also proofread the article for intellectual content. ISO carried out the electrophoresis and biochemical analysis. He also participated in revising the article for important intellectual content. ICE was involved in acquisition and interpretation of data. He also participated in revising the article critically for important intellectual content. AQK was involved in drafting the article and revising it critically for important intellectual content.

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