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Proteinuria in relation to age-dependent changes in the plasma and urine concentrations of some electrolytes and hematological indices in Wistar rats

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ABSTRACT

The study was carried out to determine the influence of proteinuria on plasma and urine concentrations of electrolytes and hematological indices in Wistar rats of different age groups.

Eighty Wistar rats of both sexes were used for this study. Groups 1 and 2 each consisted of 8 one month old male and female rats; 3 and 4 had 8 three month old rats; 5 and 6 had 8 six month old rats; 7 and 8 had 8 nine month old rats; 9 and 10 had 8 twelve month old rats.

The plasma sodium, potassium and calcium concentrations of 3 month old rats were significantly lower when compared with 1, 6, 9 and 12 months of age. Similarly, rats aged 3 months had significantly lower urine concentrations of sodium, potassium and calcium than rats of other age groups. A strong correlation was observed between the urine protein and urine sodium of the female rats at ages 3, 9 and 12 months but it was only significant at age 12 months ($p = 0.105$ and $p = 0.021$, respectively). Also, the female rats aged 3 and 12 months had a strong correlation between their urine protein and urine calcium ($p = 0.002$ and $p = 0.131$, respectively).

The red blood cells, lymphocyte and monocyte counts of the rats increased gradually and peaked at age 9 months with a subsequent decline at 12 months of age.

It was concluded that the influence of proteinuria on electrolytes was least observed in the rats aged 3 months, since they had reduced and consistent plasma and urine concentrations of electrolytes measured when compared with other age groups. This implies that long-term renal studies involving the use of rats must be carefully interpreted because of the changes in plasma and urine concentrations of electrolytes as the rats age.

1. Introduction

Proteinuria, the presence of excess serum protein in the urine has long been regarded as an important factor in the assessment of renal function. The presence of significant amount of protein in the urine of healthy Wistar rats has been reported by different researchers. These proteins are thought to be synthesized in the liver and excreted in the urine (Vettorazzi, Wait, Nagy, Monreal, & Mantle, 2013). The severity of proteinuria is associated with the rate of progression of chronic kidney disease and is a prognostic indicator in individuals with cardiac disease and diabetic nephropathies (Agodoa et al., 2001; Maschio et al., 1996)

Renal loss of plasma proteins can contribute to hypoalbuminemia; alterations in levels of coagulation factors, cellular immunity, hormonal status, mineral and electrolyte metabolism; and development of hyperlipidemia in some cases (Littman, 2011; Vaden, 2005). The plasma concentrations of some electrolytes such as Na^+, K^+ and Ca^{2+} are very important for the proper functioning of the neuromuscular and cardiovascular systems (Ganong, 2009; Guyton & Hall, 2010). Excessive loss of these ions in the urine could have deleterious consequences on these systems. For example, calcium can be bound to albumin and globulins. For each 1.0 g/dL decrease in serum albumin, total serum calcium decreases by 0.8 mg/dL. For each 1.0 g/dL decrease in serum globulin fraction, total serum calcium decreases by 0.12 mg/dL (Friedman & Gesek, 1995). This study is therefore aimed at investigating the effects of urinary protein excretion in Wistar rats at different age groups on plasma and urine electrolytes and hematological indices.

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2. Materials and methods

2.1. Ethics statement

All experimental procedures used in the study were in accordance with the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institutes of Health's (NIH) *Guide for the Care and Use of Laboratory Animals* (NIH Publications No.8023, revised 1978) and approved by Health Research and Ethics Committee of Public Health (HREC, IPH) of the Obafemi Awolowo University with reference number IPHOAU/12/919. Any rat that showed symptoms of compromised health (Inadequate movement) or had lost significant body weight was terminated according to the EU Directive 2010/63/EU on the protection of animals used for scientific research.

2.2. Animal care and management

A total of eighty (80) Wistar rats of both sexes purchased from the Animal House of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife were raised and maintained in the same laboratory where the study was carried out. The rats were housed in plastic cages and kept under natural light/dark cycle. They were allowed free access to standard rodent pellet (Ace Feed PLC Ibadan, Nigeria) and water *ad libitum*.

2.3. Experimental design

The rats were divided into ten groups of eight rats each.

1) Groups 1 and 2 consisted of 8 juveniles (one month old) male and female rats
2) Groups 3 and 4 consisted of 8 young adult (three months old) male and female rats.
3) Groups 5 and 6 consisted of 8 adults (six months old) male and female rats.
4) Groups 7 and 8 consisted of 8 aged (nine months old) male and female rats.
5) Groups 9 and 10 consisted of 8 aged (twelve months old) male and female rats.

The rats were allowed to acclimatize for one week in separate metabolic cages (fabricated by Central Technological Laboratory and Workshops (CTLW), OAU, Ile-Ife, Nigeria, according to Ohaus R Model; Ohaus, Pine Brook, New Jersey., USA) before the commencement of urine collection. Urine samples of rats were collected over a 24-hour period at the end of attainment of the chosen ages. Thereafter, they were anaesthetized with ketamine hydrochloride (10 mg/kg; Rotex Medica, Trittau, Germany) through intramuscular route. Blood from each rat was collected by cardiac puncture into separate potassium-EDTA and fluoride oxalate bottles. Blood dispensed into potassium-EDTA bottles was used for hematological analysis while that collected in fluoride oxalate bottles was centrifuged at 4000 rpm for 15 min at –4 °C using a cold centrifuge (Centurium Scientific, Model 8881) to obtain the plasma.

2.4. Hematological analysis

The full and differential blood counts were determined using an auto-analyzer machine (SFRI Blood Cell Counter, H18 Light, France).

2.5. Biochemical assay

Plasma and urine total protein was determined according to the method of (Lowry, Rosebrough, Farr, & Randall, 1951).

2.6. Electrolyte assay

The urine and plasma concentrations of sodium, potassium and calcium were determined using PG 990 Atomic Absorbance Spectrophotometer (AAS) of the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, while phosphate and chloride levels were assessed using ion-selective electrode.

2.7. Statistical analysis

The results obtained were expressed as mean ± SEM. Comparison between the age groups were carried out using one-way ANOVA followed by Newman-Keuls multiple comparison test as the post hoc analysis (Graph Pad Software Inc., CA, USA). Student's t-test was used for paired observation. The results were considered significant when $p < 0.05$. Correlation analyses were done using Pearson's r coefficient for continuous variables.

2.8. Histological processes

The kidneys of the rats were fixed in 10% formo-saline. Sections were deparaffinized and rehydrated in descending grades of alcohol. They were then oxidized in 0.5% periodic acid solution for 5 min, thereafter they were rinsed in distilled water to remove excess acid and placed in Schiff's reagent for 15 min (sections turned light pink). Sections were washed in lukewarm water for 5 min (immediately sections turned dark pink). Afterward, they were counterstained in Mayer's hematoxylin for 1 min, washed in tap water for 5 min and then dehydrated and cover slipped using a synthetic mounting medium. Sections were examined under a light microscope (Olympus CH; Olympus, Tokyo, Japan) with photomicrographs taken with a Leica DM750 Camera Microscope at x 400 magnification. Glycogen and other periodate reactive carbohydrates stained magenta while nuclei stained blue.

3. Results

3.1. Age – related differences in plasma and urine concentrations of total protein in male Wistar rats

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

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 that of rats at ages 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 at age 6 months (Fig. 2).

3.2. Age – related differences in the plasma and urine concentrations of total protein in female Wistar rats

Also, 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 (Fig. 3).

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 of ages 6, 9 and 12 months ($F = 16.47$; $p = 0.0001$) (Fig. 4).

3.3. Age – related differences in plasma concentrations of some electrolytes in male Wistar rats

The plasma potassium concentrations of 3 and 9 month old rats was significantly lower ($F = 242.5$; $p = 0.0001$) when compared with rats of ages 1, 6 and 12 months. Also, 6 and 12 month old rats had

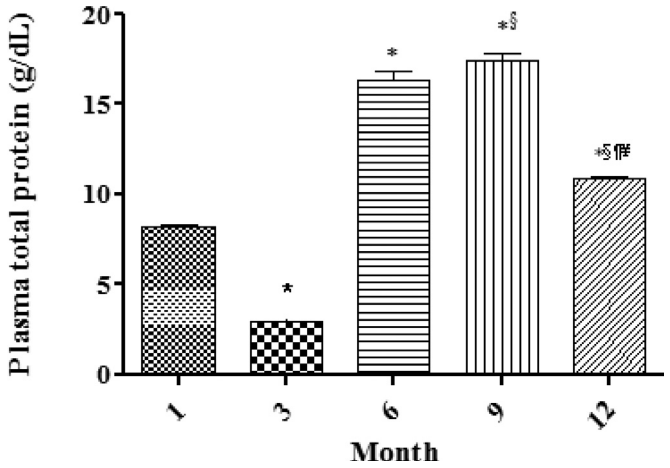

Fig. 1. Age – related differences in urine total protein of male Wistar rats. Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$. * = Significantly different from 1 month old rats. § = Significantly different from 3 month old rats. ¶ = Significantly different from 6 month old rats. ¥ = Significantly different from 9 month old rats.

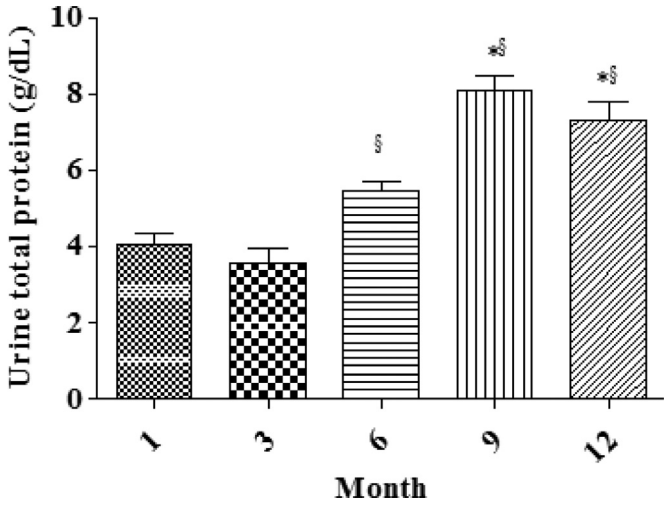

Fig. 2. Age – related differences in urine total protein of male Wistar rats. Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$. * = Significantly different from 1 month old rats. § = Significantly different from 3 month old rats. ¶ = Significantly different from 6 month old rats. ¥ = Significantly different from 9 month old rats.

significantly higher plasma potassium concentrations than that of rats age 1 month ($F = 167.3$; $p = 0.0001$).

The plasma sodium concentration of 1 month old rats was significantly higher ($F = 15.27$; $p = 0.002$) than that of 3 and 6 month old rats but significantly lower ($F = 354.3$; $p = 0.0001$) when compared with 9 and 12 month old rats. Also, 9 and 12 month old rats had plasma sodium concentrations that were significantly higher ($F = 306.9$; $p = 0.0001$) than that of 3 and 6 month old rats.

Rats at age 1 month had plasma calcium concentration that was significantly higher ($t = 5.453$; $p = 0.0001$) than that of 3 months but significantly lower ($F = 26.74$; $p = 0.0001$) when compared with 6, 9 and 12 month old rats. However, the plasma calcium concentration of 3 month old rats was significantly lower ($F = 184.4$; $p = 0.0001$) when compared with 6, 9 and 12 month old rats.

A significantly higher plasma phosphate concentration was observed in rats at 1 month of age when compared with 3 month old rats ($t = 4.176$; $p = 0.001$) but significantly lower ($F = 6.970$; $p = 0.001$) than that of 6, 9 and 12 months old rats (Table 1).

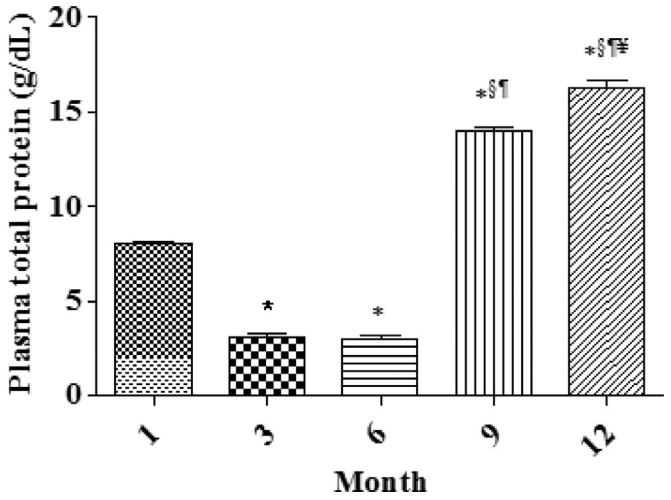

Fig. 3. Age – related differences in plasma total protein of female Wistar rats. Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$. * = Significantly different from 1 month old rats. § = Significantly different from 3 month old rats. ¶ = Significantly different from 6 month old rats. ¥ = Significantly different from 9 month old rats.

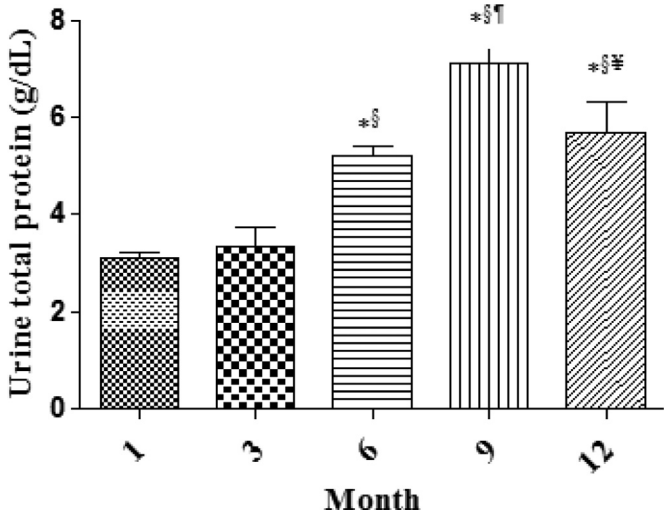

Fig. 4. Age – related differences in urine total protein of female Wistar rats. Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$. * = Significantly different from 1 month old rats. § = Significantly different from 3 month old rats. ¶ = Significantly different from 6 month old rats. ¥ = Significantly different from 9 month old rats.

3.4. Age – related differences in urine concentrations of some electrolytes in male Wistar rats

The urine potassium concentrations of 3 and 9 month old rats were significantly lower ($F = 72.74$; $p = 0.0001$) when compared with 1, 6 and 12 month old rats. On the other hand, rats aged 1 and 6 months had urine potassium concentrations that were significantly lower ($F = 418.0$; $p = 0.0001$) than that of 12 month old rats.

The urine sodium concentrations of 3 and 6 month old rats were significantly lower ($F = 813.9$; $p = 0.0001$) when compared with rats of ages 1, 9 and 12 months. Rats at 9 months of age had urine sodium concentration that was significantly higher ($F = 642.1$; $p = 0.0001$) than that of 1 and 12 month old rats.

A significantly lower urine calcium concentration was recorded in the 3 month old rats when compared with rats of other age groups ($F = 189.0$; $p = 0.0001$). Also, male rats at 1 month of age had urine calcium concentration that was significantly higher ($F = 3.912$;

Table 1
Age – related changes in plasma and urine concentrations of some electrolytes in male Wistar rats.

	Age (Month)				
Plasma	1	3	6	9	12
K^+ (mg/L)	0.62 ± 0.03	[a]0.20 ± 0.02	[ab]0.93 ± 0.02	[ac]0.22 ± 0.02	[abcd]1.62 ± 0.07
Na^+ (mg/L)	1.85 ± 0.14	[a]0.98 ± 0.25	[a]0.59 ± 0.01	[abc]6.70 ± 0.11	[abcd]3.15 ± 0.12
Ca^{2+} (mg/L)	0.61 ± 0.06	[a]0.29 ± 0.02	[ab]0.85 ± 0.02	[ab]0.82 ± 0.03	[abcd]1.09 ± 0.04
$P0_4^{3-}$ (mg/L)	1.64 ± ± 0.32	[a]0.41 ± 0.03	[ab]3.14 ± 0.60	[ab]3.73 ± 0.89	[ab]3.74 ± 0.70
Urine					
K^+ (mg/L)	0.86 ± 0.03	[a]0.15 ± 0.02	[b]1.03 ± 0.01	[c]0.47 ± 0.21	[abcd]2.23 ± 0.06
Na^+ (mg/L)	2.04 ± 0.04	[a]0.53 ± 0.12	[a]0.69 ± 0.02	[abc]8.06 ± 0.18	[abcd]3.91 ± 0.09
Ca^{2+} (mg/L)	1.09 ± 0.04	[a]0.15 ± 0.01	[ab]0.94 ± 0.05	[b]1.04 ± 0.03	[abcd]1.37 ± 0.04
$P0_4^{3-}$ (mg/L)	2.70 ± 0.55	[a]0.77 ± 0.04	[b]3.89 ± 0.62	[b]4.17 ± 0.95	[ab]5.65 ± 0.98
Urine Volume (mL)	1.63 ± 0.29	2.17 ± 0.31	2.33 ± 0.33	1.52 ± 0.35	§¶1.35 ± 0.25
Urine Volume (mL)	1.28 ± 0.14	1.12 ± 0.08	1.40 ± 0.21	1.89 ± 0.29	*§¶2.87 ± 0.26

Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$.
[a] Significantly different from 1 month old rats.
[b] Significantly different from 3 month old rats.
[c] Significantly different from 6 month old rats.
[d] Significantly different from 9 month old rats. K^+ = Potassium; Na^+ = Sodium; Ca^{2+} = Calcium; PO_4^{3-} = Phosphate.

$p = 0.043$) than that of 6 and 9 month old rats but significantly lower ($t = 4.855$; $p = 0.0003$) when compared with rats at age 12 months.

The phosphate concentration of rats at age 3 months was significantly lower ($F = 7.228$; $p = 0.0004$ and $F = 47.75$; $p = 0.0001$, respectively) when compared with rats of other age groups. Rats aged 12 months had urine phosphate concentration that was significantly higher ($t = 2.627$; $p = 0.0013$) when compared with 1 month old rats.

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 (Table 1).

3.5. Age – related differences in plasma concentrations of some electrolytes in female Wistar rats

At 3 months of age, plasma potassium concentration was significantly lower ($F = 162.0$; $p = 0.0001$) when compared with 1, 6, 9 and 12 month old rats. A significantly higher plasma potassium concentration was recorded in the 12 month old rats when compared with rats at ages 1, 6 and 9 months ($F = 162.0$; $p = 0.0001$). Also, the plasma potassium concentration of rats aged 1 month was significantly lower ($F = 74.75$; $p = 0.0001$) when compared with 6 and 9 month old rats.

The plasma sodium concentration in rats at 3 months of age was significantly lower ($F = 297.4$; $p = 0.0001$) when compared with rats of ages 1, 6, 9 and 12 months. Rats aged 1 month had plasma sodium concentration that was significantly higher ($t = 9.665$; $p = 0.0001$) than that of 6 month old rats but significantly lower ($F = 203.4$; $p = 0.0001$) when compared with 9 and 12 month old rats. The plasma sodium concentration of 6 month old rats was significantly lower ($F = 501.3$; $p = 0.0001$) when compared with 9 and 12 month old rats.

In comparison with 3 month old rats, plasma calcium concentration was significantly lower ($F = 105.7$; $p = 0.0001$) in rats of ages 1, 6 and 9 months. The 1 month old rats, however, had plasma calcium level that was significantly below the values ($F = 51.72$; $p = 0.0001$) that were observed in 6 and 9 months old rats. The plasma calcium concentration of rats aged 12 months was significantly higher ($F = 57.01$; $p = 0.0001$) than that of ages 6 and 9 months.

In 1 and 3 month old rats, plasma phosphate concentration was significantly lower ($F = 6.658$; $p = 0.001$) when compared with 6, 9 and 12 month old rats (Table 2).

3.6. Age – related differences in urine concentrations of some electrolytes in female Wistar rats

A significantly lower urine potassium concentration was recorded in 3 month old rats in comparison with rats of other age groups ($F = 188.9$; $p = 0.0001$). At 1 month of age, the urine potassium concentration was significantly lower ($F = 45.93$; $p = 0.0001$) when compared with 9 and 12 month old rats. The female rats at 9 and 12 months of age had significantly higher urine potassium concentrations when compared with 6 month old rats ($F = 196.0$, $p = 0.0001$).

The urine sodium concentration of 1 month old rats was significantly higher ($F = 27.30$; $p = 0.0001$) than that of 3 and 6 month old rats and significantly lower ($F = 676.1$; $p = 0.0001$) when compared with the 9 and 12 month old rats. In 3 and 6 month old rats, urine sodium concentrations were significantly lower ($F = 561.0$; $p = 0.0001$) when compared with 9 and 12 month old rats.

The urine calcium concentration of rats age 3 months was significantly lower ($F = 141.2$; $p = 0.0001$) when compared with rats of other age groups. At 6 month of age, urine calcium concentration was significantly lower ($F = 45.49$; $p = 0.0001$) than that of rats aged 9 and 12 months. The urine calcium concentration of rats aged 1 month was significantly lower ($F = 34.18$; $p = 0.0001$) when compared with 9 and 12 month old rats. Also, urine calcium concentration of 12 month old rats was significantly higher ($F = 5.982$; $p = 0.0001$) than that of 9 month old rats.

The urine phosphate concentrations of 6, 9 and 12 month old rats were significantly higher ($F = 7.521$; $p = 0.0004$) when compared with rats of 1 and 3 months of age.

The urine volume of 12 month old female rats was significantly higher ($F = 7.402$; $p = 0.0003$) when compared with 1, 3 and 6 months old rats (Table 2).

3.7. Age – related differences in hematological indices of male Wistar rats

The percentage lymphocyte counts of rats aged 9 months was significantly lower ($F = 7.563$; $p = 0.0004$) when compared with rats of other age groups. Also, the percentage monocyte counts of rats aged 6 and 9 months were significantly higher ($F = 4.858$; $p = 0.005$) than that of 1 month old rats. Significantly lower percentage granulocyte counts were observed in rats at 3, 6 and 12 months of age when compared with 9 month old rats ($F = 9.516$; $p = 0.0001$). Also, the percentage granulocyte counts of rats age 12 months was significantly lower ($F = 9.516$; $p = 0.0001$) than that of 1 month old rats.

Rats at ages 6 and 9 months had monocyte counts that were significantly higher ($F = 8.718$; $p = 0.0002$) than that of 1 and 3 month old rats. The granulocyte counts of rats aged 9 months was significantly higher ($F = 7.644$; $p = 0.0004$) when compared other age groups.

The red blood cell count of 9 month old rats was significantly higher ($F = 16.77$; $p = 0.0001$) when compared with rats of other age groups.

Table 2
Age – related changes in plasma and urine concentrations of some electrolytes in female Wistar rats.

	Age (Month)				
Plasma	1	3	6	9	12
K^+ (mg/L)	0.63 ± 0.03	[a]0.14 ± 0.02	[ab]0.98 ± 0.03	[abc]1.11 ± 0.03	[abcd]1.27 ± 0.06
Na^+ (mg/L)	1.18 ± 0.07	[a]0.33 ± 0.08	[a]0.45 ± 0.02	[abc]1.83 ± 0.04	[abcd]3.26 ± 0.09
Ca^{2+} (mg/L)	0.56 ± 0.05	[a]0.14 ± 0.03	[ab]0.79 ± 0.04	[abc]1.14 ± 0.04	[bd]0.64 ± ± 0.03
$P0_4^{3-}$ (mg/L)	0.49 ± 0.09	0.62 ± 0.04	[ab]2.97 ± 0.72	[ab]3.01 ± 0.68	[ab]2.84 ± 0.55
Urine					
K^+ (mg/L)	1.05 ± 0.08	[a]0.15 ± 0.03	[ab]0.76 ± 0.02	[bc]1.21 ± 0.01	[abcd]1.68 ± 0.05
Na^+ (mg/L)	1.36 ± 0.06	[a]0.57 ± 0.15	[a]0.38 ± 0.01	[abc]2.17 ± 0.04	[abcd]4.65 ± 0.08
Ca^{2+} (mg/L)	0.88 ± 0.04	[a]0.15 ± 0.01	[b]0.78 ± 0.05	[abc]1.02 ± 0.04	[acd]1.31 ± 0.03
$P0_4^{3-}$ (mg/L)	0.87 ± 0.19	0.89 ± 0.01	[ab]3.75 ± 0.74	[ab]3.99 ± 0.64	[ab]4.18 ± 0.77
Urine Volume (mL)	1.63 ± 0.29	2.17 ± 0.31	2.33 ± 0.33	1.52 ± 0.35	§¶1.35 ± 0.25
Urine Volume (mL)	1.28 ± 0.14	1.12 ± 0.08	1.40 ± 0.21	1.89 ± 0.29	*§¶2.87 ± 0.26

Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$.
[a] Significantly different from 1 month old rats.
[b] Significantly different from 3 month old rats.
[c] Significantly different from 6 month old rats.
[d] Significantly different from 9 month old rats. K^+ = Potassium; Na^+ = Sodium; Ca^{2+} = Calcium; PO_4^{3-} = Phosphate.

Rats aged 12 months had red blood cell count that was significantly lower than that of 3 and 6 month old rats. Hemoglobin concentration of rats aged 9 months was significantly higher ($F = 14.32$; $p = 0.0001$) than that of 1, 3, 6 and 12 month old rats. Rats aged 6 months had hemoglobin concentration that was significantly higher than that of 3 month old rats. Hematocrit of 9 month old rats was significantly higher ($F = 38.50$; $p = 0.0001$) that other age groups. Rats aged 1, 3 and 6 months had hematocrits that were significantly higher ($F = 38.50$; $p = 0.0001$) than that of age 12 months. The mean corpuscular volumes of rats aged 6, 9 and 12 months were significantly lower ($F = 18.53$; $p = 0.0001$) than that of rats aged 1 and 3 months.

Significantly higher mean corpuscular hemoglobin counts were recorded in rats of ages 6, 9 and 12 when compared with rats of 3 months of age ($F = 8.455$; $p = 0.0002$). The mean corpuscular hemoglobin concentration of 1 month old rats was significantly higher ($F = 56.20$; $p = 0.0001$) when compared with 3 month old rats but significantly lower ($F = 56.20$; $p = 0.0001$) than that of 6, 9 and 12 month old rats. Also, rats aged 12 months had mean corpuscular hemoglobin concentration that was significantly higher ($F = 56.20$; $p = 0.0001$) than that of ages 3, 6 and 9 months. The platelet counts of 9 and 12 month old rats were significantly higher ($F = 18.98$; $p = 0.0001$) when compared with 1, 3 and 6 month old rats (Table 3).

3.8. Age – related differences in hematological indices of female Wistar rats

The white blood count of 1 month old rats was lower than that of 3, 6, 9 and 12 month old rats, although it was not significantly different ($F = 1.366$; $p = 0.274$) from other age groups.

Rats aged 12 months had percentage monocyte count that was significantly higher ($F = 6.199$; $p = 0.001$) than rats of ages 1, 3 and 6 months. The monocyte and lymphocyte counts of rats aged 1 month were lower when compared with rats of other age groups but they were not significantly different ($F = 1.975$; $p = 0.128$ and $F = 1.116$; $p = 0.370$, respectively) from them.

Rats aged 9 months had red blood cell count that was lower but not significantly different ($F = 1.498$; $p = 0.232$) from other age groups. A significantly lower hemoglobin concentration was recorded in 3 month old rats when compared with rats of 1 and 9 months of age ($F = 3.810$; $p = 0.014$). The hematocrit of 6 month old rats was lower than that of the other age groups but it was only significant ($F = 5.211$; $p = 0.003$) when compared with the 3 month old rats.

Rats of ages 6 and 9 months had significantly lower mean

Table 3
Age – related differences in hematological indices of male Wistar rats.

Parameter	1	3	6	9	12
WBC (μL)	3.04 ± 0.29	3.70 ± 0.26	3.62 ± 0.67	4.30 ± 0.39	3.12 ± 0.28
LYM%	70.78 ± 1.85	73.76 ± 1.28	71.7 ± 0.93	[abc]62.23 ± 2.10	[d]73.32 ± 2.21
MON%	10.04 ± 0.88	10.90 ± 0.41	[a]13.83 ± 1.01	[a]14.33 ± 0.95	13.43 ± 0.86
GRAN%	19.18 ± 1.69	15.44 ± 0.49	15.00 ± 0.82	[bc]23.43 ± 1.78	[ad]13.25 ± 1.41
LYM(μL)	2.16 ± 0.25	2.79 ± 0.17	3.05 ± 0.23	2.68 ± 0.29	2.27 ± 0.18
MON (μL)	0.30 ± 0.04	0.40 ± 0.03	[ab]0.58 ± 0.04	[ab]0.60 ± 0.04	0.43 ± 0.06
GRAN (μL)	0.58 ± 0.05	0.59 ± 0.05	0.65 ± 0.08	[abc]1.02 ± 0.12	[d]0.43 ± 0.08
RBC (μL)	7.25 ± 0.17	7.35 ± 0.06	7.36 ± 0.09	[abc]8.06 ± 0.09	[bcd]6.93 ± 0.10
HGB (g/dL)	16.34 ± 0.46	15.21 ± 0.16	[b]16.68 ± 0.32	[abc]18.10 ± 0.31	[d]15.78 ± 0.25
HCT (%)	42.66 ± 1.09	45.76 ± 0.57	44.07 ± 0.84	[abc]49.83 ± 1.54	[abcd]33.73 ± 0.57
MCV (fL)	58.86 ± 0.26	62.81 ± 0.85	[ab]53.45 ± 0.66	[ab]52.80 ± 0.97	[ab]50.40 ± 2.22
MCH (pg)	22.46 ± 0.16	[a]20.67 ± 0.18	[b]22.63 ± 0.34	[b]22.42 ± 0.34	[b]22.75 ± 0.44
MCHC (g/dL)	38.26 ± 0.19	[a]33.23 ± 0.49	[ab]42.45 ± 0.89	[abb]42.60 ± 0.99	[abcd]46.77 ± 0.62
PLT (μL)	489.8 ± 36.75	705.2 ± 35.84	545.2 ± 56.47	[abc]1048 ± 93.39	[abc]1003 ± 42.46
MPV (fL)	6.70 ± 0.05	6.94 ± 0.21	7.77 ± 0.63	7.23 ± 0.39	7.05 ± 0.09

Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$.
[a] Significantly different from 1 month old rats.
[b] Significantly different from 3 month old rats.
[c] Significantly different from 6 month old rats.
[d] Significantly different from 9 month old rats. WBC = White blood cells; LYM = Lymphocyte; MON = Monocyte; GRAN = Granulocyte; RBC = Red blood cell; HGB = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; MCV = Mean corpuscular volume; PLT = Platelet; MPV = Mean platelet volume.

Table 4
Age – related differences in hematological indices of female Wistar rats.

Parameter	1	3	6	9	12
WBC (μL)	2.66 ± 0.11	3.23 ± 0.24	3.78 ± 0.53	3.47 ± 0.31	3.45 ± 0.33
LYM%	70.02 ± 1.55	73.17 ± 1.95	73.85 ± 0.69	67.58 ± 3.01	69.77 ± 1.65
MON%	8.72 ± 0.23	9.77 ± 0.65	10.25 ± 0.28	11.00 ± 1.21	[abc]12.97 ± 0.28
GRAN%	21.26 ± 1.72	17.70 ± 1.58	15.90 ± 0.61	21.42 ± 1.99	17.30 ± 1.61
LYM (μL)	1.86 ± 0.07	2.46 ± 0.18	2.80 ± 0.39	2.33 ± 0.17	2.31 ± 0.22
MON (μL)	0.24 ± 0.03	0.34 ± 0.04	0.38 ± 0.05	0.42 ± 0.08	0.43 ± 0.04
GRAN (μL)	0.56 ± 0.07	0.56 ± 0.06	0.60 ± 0.09	0.72 ± 0.10	0.50 ± 0.06
RBC (μL)	7.17 ± 0.13	7.24 ± 0.13	7.29 ± 0.14	6.80 ± 0.13	7.02 ± 0.21
HGB (g/dL)	16.56 ± 0.31	[a]14.67 ± 0.42	15.40 ± 0.33	[b]16.23 ± 0.29	15.47 ± 0.44
HCT (%)	42.40 ± 0.59	45.07 ± 1.06	[b]38.68 ± 0.79	42.33 ± 0.88	41.21 ± 1.35
MCV (fL)	59.24 ± 0.58	62.43 ± 0.41	[ab]53.22 ± 1.27	[ab]55.50 ± 0.81	[bc]58.81 ± 0.80
MCH (pg)	23.04 ± 0.24	[a]21.00 ± 0.26	[a]21.07 ± 0.22	[bc]23.80 ± 0.15	[bd]22.00 ± 0.32
MCHC (g/dL)	39.00 ± 0.28	[a]33.67 ± 0.30	[b]39.85 ± 1.29	[abc]43.05 ± 0.54	[bd]37.53 ± 0.46
PLT (μL)	617.2 ± 82.94	650.2 ± 41.47	612.0 ± 88.77	491.7 ± 58.89	792.0 ± 46.89
MPV (fL)	7.12 ± 0.12	7.00 ± 0.14	7.35 ± 0.52	6.95 ± 0.13	6.80 ± 0.07

Values are expressed as mean ± SEM ($n = 8$). $p < 0.05$. Significantly different from 1 month old rats. Significantly different from 3 month old rats. Significantly different from 6 month old rats. Significantly different from 9 month old rats. WBC = White blood cells; LYM = Lymphocyte; MON = Monocyte; GRAN = Granulocyte; RBC = Red blood cell; HGB = Hemoglobin; HCT = Hematocrit; MCV = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; MCV = Mean corpuscular volume; PLT = Platelet; MPV = Mean platelet volume.

corpuscular volumes when compared with 1 and 3 month old rats ($F = 19.40$; $p = 0.0001$).

The mean corpuscular hemoglobin of rats aged 3 and 6 months were significantly lower ($F = 22.67$; $p = 0.0001$) than that of ages 1, 9 and 12 months. Also, the mean corpuscular hemoglobin of 12 month old rats was significantly lower ($F = 22.67$; $p = 0.0001$) than that of rats aged 9 months. The mean corpuscular hemoglobin concentration of rats aged 1 month was significantly higher ($F = 27.24$; $p = 0.0001$) when compared with 3 month old rats and significantly lower than that of 9 month old rats.

Also, rats of ages 6, 9 and 12 months had significantly higher mean corpuscular hemoglobin concentrations when compared with rats of age 3 months ($F = 27.24$; $p = 0.0001$). The mean corpuscular hemoglobin concentration of 9 month old rats was significantly higher ($F = 27.24$; $p = 0.0001$) than that of rats aged 6 and 12 months. The platelet counts of 9 month old rats was significantly lower ($F = 2.792$; $p = 0.049$) than rats of age 12 months (Table 4).

3.9. Correlation between urine protein and urine concentrations of electrolytes of Wistar rats

There was a strong negative correlation between the urine protein and urine sodium of the male rats at 1 month of age. However, a weak negative correlation existed between the urine protein and urine sodium of the male rats at ages 3 and 12 months, while no linear correlation was found between the urine protein and urine sodium of 9 month old rats (Table 5).

A strong negative correlation was observed between the urine protein and urine sodium of the female rats at ages 3, 9 and 12 months but it was only significant at age 12 months. Rats aged 1 and 6 months had weak negative and positive correlations between their urine protein and urine sodium (Table 6).

There was a weak negative correlation between the urine protein and urine potassium of the male rats at ages 1 and 9 months. No linear correlation was observed between the urine protein and urine potassium of the male rats ages 3 and 12 months. However, a moderate negative correlation was observed between the urine protein and urine potassium of the 6 month old rats (Table 5).

No linear correlation existed between the urine protein and urine potassium of the female rats at the different ages under study, except for the female rats aged 9 months which had a weak correlation (Table 6).

The male rats aged 1 and 3 months had moderate positive correlation between their urine protein and urine calcium. Also, a moderate negative correlation was found between the urine protein and urine calcium of the male rats at age 6 months. No linear correlation existed between the urine protein and urine calcium of the male rats at ages 9 and 12 months (Table 5).

A weak negative correlation was found between the urine protein and urine calcium of the female rats at 1 and 6 months of age. However, a strong positive correlation was observed between the urine protein and urine calcium of rats aged 3 and 12 months. There was no linear association between the urine protein and urine calcium of female rats aged 6 months (Table 6).

There was no linear correlation between the urine protein and urine phosphate of the male rats at the different ages under study (Table 5).

The female rats aged 9 months had strong positive correlation between their urine protein and urine phosphate. Also, a moderate positive and negative correlation was found between the urine protein and urine phosphate of the female rats aged 1 and 12 months. A moderate negative correlation existed between the urine protein and urine phosphate of the female rats aged 3 months. No linear correlation was found between the urine protein and urine phosphate of the 6 month old female rats (Table 6).

Table 5
Correlation between urine protein and urine concentrations of some electrolytes in male Wistar rats.

Age (Month)	1		3		6		9		12	
Urine	r	p	r	p	r	p	r	p	r	p
Na^+	−0.817	0.092	−0.195	0.712	−0.591	0.218	0.131	0.805	−0.256	0.625
K^+	−0.244	0.692	0.054	0.919	−0.463	0.355	−0.294	0.572	−0.160	0.762
Ca^{2+}	0.576	0.309	0.650	0.162	−0.467	0.350	0.011	0.983	−0.199	0.705
PO_4^{3-}	0.051	0.935	−0.053	0.921	0.221	0.674	−0.276	0.597	0.045	0.931

Correlation is significant at $p < 0.05$ (2-tailed).

Table 6
Correlation between urine protein and urine concentrations of some electrolytes in female Wistar rats.

Age (Month)										
Urine	1		3		6		9		12	
	r	P	r	p	r	p	r	p	r	p
Na^+	−0.235	0.704	−0.722	0.105	0.227	0.665	0.855	*0.014	−0.829	*0.021
K^+	0.191	0.758	−0.112	0.833	0.219	0.676	−0.332	0.407	0.069	0.802
Ca^{2+}	−0.226	0.715	0.964	*0.002	0.181	0.731	−0.403	0.369	0.628	0.131
PO_4^{3-}	−0.588	0.291	−0.457	0.362	−0.199	0.705	0.711	0.073	0.597	0.157

Correlation is significant at $p < 0.05$ (2-tailed).

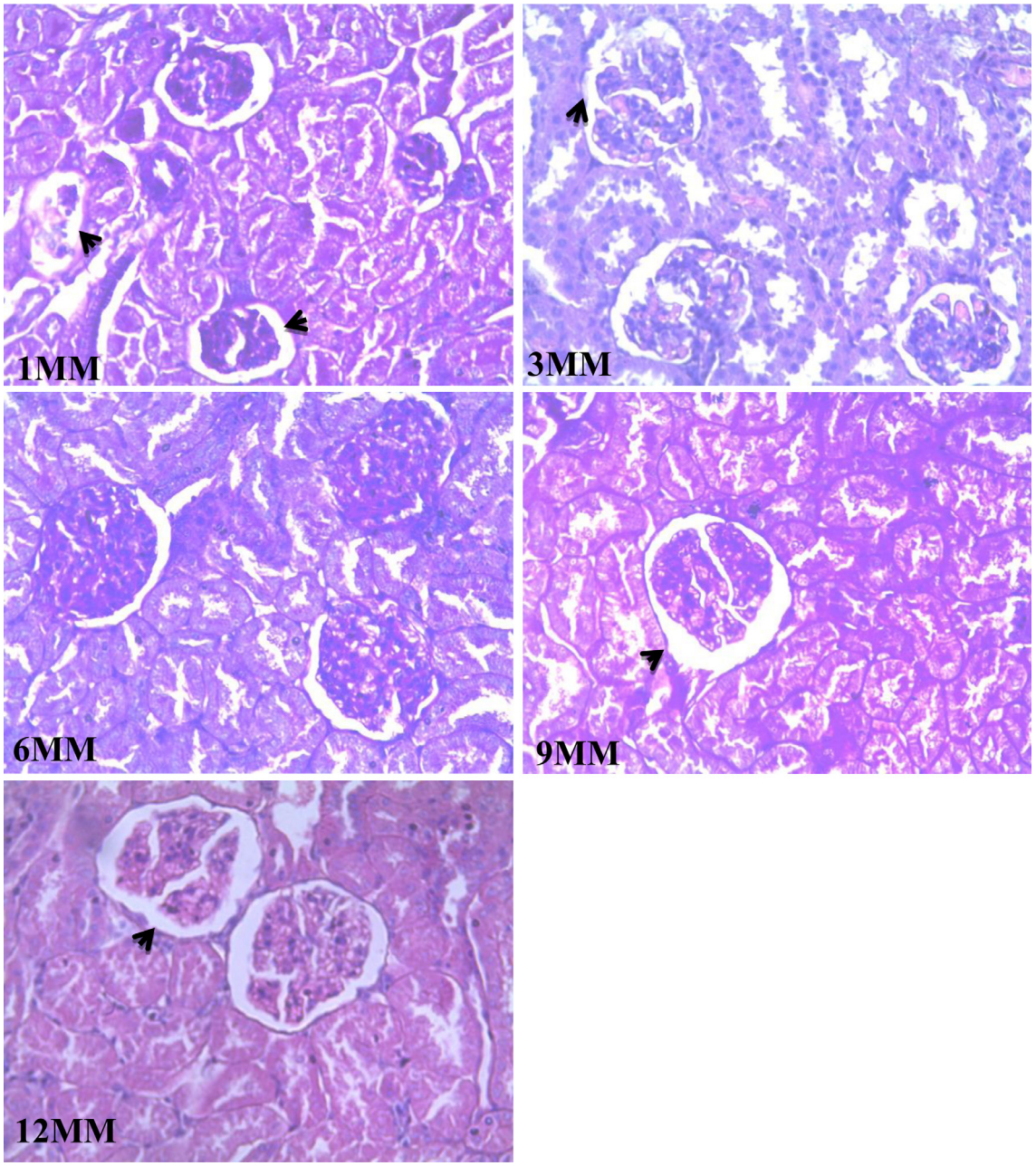

Fig. 5. Photomicrographs of the male kidney cortex (1 MM; 1-month male rats, 3 MM; 3-month male rats, 6 MM; 6-month male rats, 9 MM; 9-month male rats, 12 MM; 12-month male rats. The basement membrane supporting the glomerular epithelium was moderately PAS-positive (black arrow). The endothelial cells and podocytes of glomerulus are normal, with no necrosis seen. Stain PAS. Mag X 400.

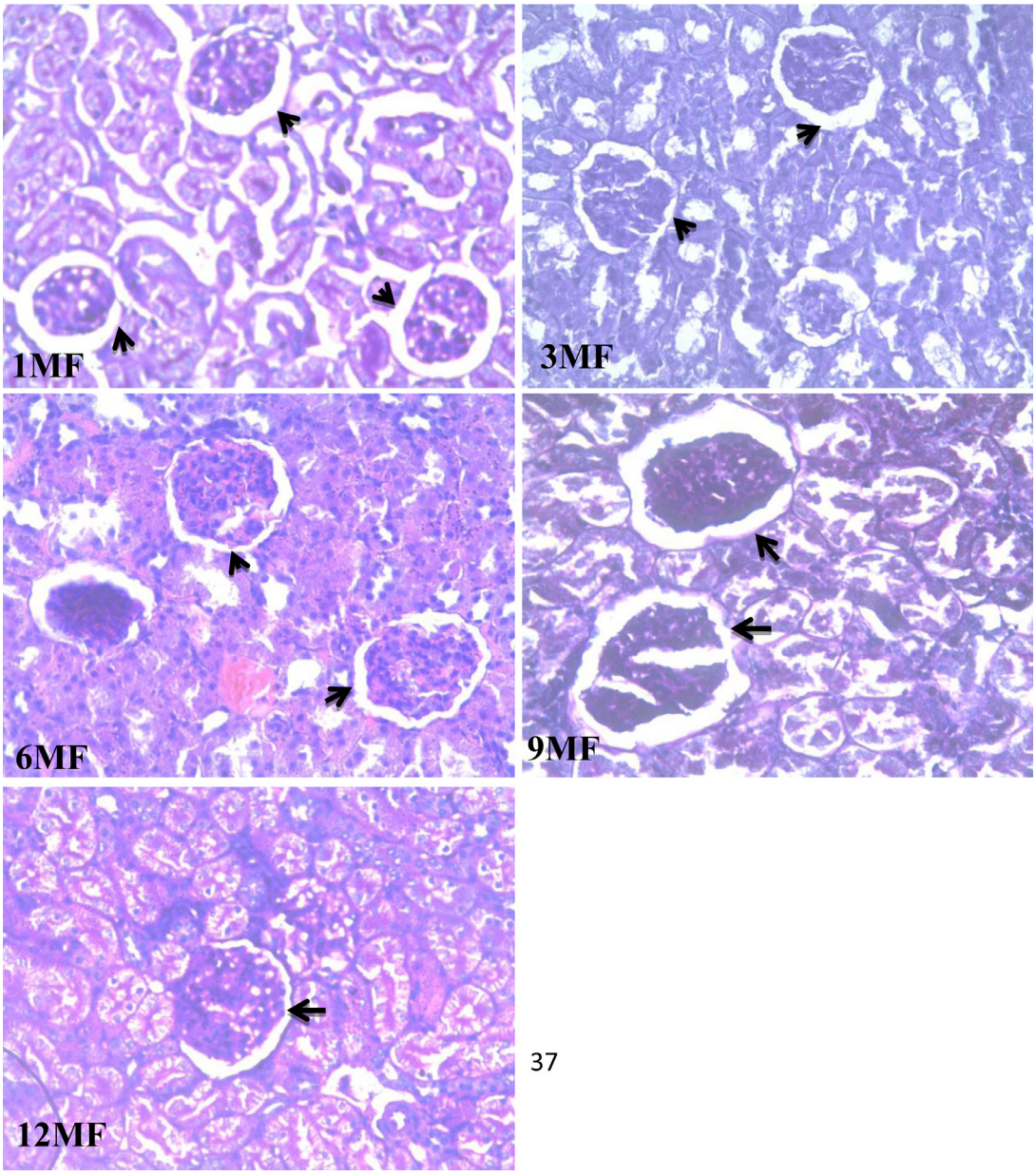

Fig. 6. Photomicrographs of the female kidney cortex (1MF; 1-month male rats, 3MF; 3-month male rats, 6MF; 6-month male rats, 9MF; 9-month male rats, 12MF; 12-month male rats. The photomicrographs show moderate intensity of PAS stain at the basement membrane of the bowman's capsule (black arrow). The endothelial cells and podocytes appear normal across the age groups. Stain PAS. Mag X 400.

3.10. Photomicrographs of the kidney cortex of male and female Wistar rats

The photomicrographs of the male and female kidney cortex show that the basement membrane supporting the glomerular epithelium was moderately PAS-positive. The endothelial cells and podocytes of glomerulus are normal, with no necrosis seen (PAS) (Figs. 5 and 6).

4. Discussion

The study investigated the influence of urinary protein excretion in Wistar rats at different age groups on plasma and urine electrolytes and hematological indices. Aldosterone secreted from the adrenal cortex is an important regulatory factor of renal potassium excretion and changes in plasma aldosterone influence the potassium balance by its action on the collecting duct. Araujo, Helou, and Seguro (1998) showed that aging rats have lower plasma renin activity and aldosterone concentration than young rats. Weinstein and Anderson (2010) also reported that aldosterone secretion decreases with aging due to decreased renin production and release. The plasma potassium concentrations of 3 and 9 month old rats were significantly lower than that of other age groups. Also, the urine potassium concentration of the male rats at 3 months of age was significantly lower than that of other age groups. The increase in the urine potassium concentration in the 9 and 12 month old rats could not have been due to hormonal influence, since aldosterone has been reported to decrease with aging, but rather to the compensating mechanism by the kidney to concentrate urine in a state of fluid loss or reduced water content in the body. The percentage of total body weight that is fluid gradually decreases with aging. Increased extracellular fluid osmolarity as a result of reduced water content causes osmotic flow of water out of the cells. The resultant cellular dehydration increases intracellular potassium concentration, thereby promoting diffusion of potassium out of the cells and increasing extracellular fluid potassium concentration (Guyton & Hall, 2010).

The secretion of antidiuretic hormone (ADH) from the posterior

pituitary is an important homeostatic mechanism. The studies of Bengele, Mathias, Perkins, and Alexander (1981b) indicated that the collecting ducts of aged rats were less permeable to water than those of young rats, even when normal circulating antidiuretic hormone levels were maintained. The insight into the nature of this defect were given by the finding of a significant reduction in generation of vasopressin-dependent cAMP in the papillae of 24- month old Fischer 344 rats (Beck & Yu, 1982). The plasma sodium concentration of 9 and 12 month old rats was significantly higher when compared with other age groups. This may not have resulted from excess loss of water from the extracellular fluid, which concentrates the sodium ions, since the urine output of these age groups was significantly lower than that of 1, 3 and 6 month old rats, but from reduced water content in the body as mentioned earlier and/or increased secretion of angiotensin II.

Angiotensin II secretion at the renal parenchyma is markedly increased in aging rats despite the decrease in renin - angiotensin- aldosterone system activity. This has been attributed to the histological renal modifications induced by the senile glomerulosclerosis process and also associated with the vascular hemodynamic alterations suffered by the aged kidney (Musso & Oreopoulos, 2011). Angiotensin II stimulates vasoconstriction of the efferent arteriole, which in turn increases reabsorption of sodium by the proximal tubule by decreasing blood flow through the peritubular capillaries (Musso, Alvarez, & Herrera Perez del Villar, 2011).

The collecting duct is normally responsible for the final control of urinary sodium and potassium excretion. The reabsorption of sodium and secretion of potassium are stimulated by aldosterone, which induces an increase in the number and activity of the apical Na^+ and K^+ channels as well as in the basolateral Na^+– K^+ ATPase pumps and apical H^+ ATPase pumps (Foster, Macfarlane, & Bustamante, 1997; Palmer, 2015). Thus, Na^+ is actively transported out of the cell of the renal tubule into the interstitium by Na^+- K^+ATPase. This generates a negative electrical potential in the lumen responsible for stimulating $K^+$and H^+ secretion (Renkke & Denker, 2007). In this study, the urine sodium concentrations of the rats aged 9 and 12 months were significantly higher when compared with 3 and 6 month old rats. This could have been due to the reduction of sodium reabsorption by the collecting ducts of the rats resulting from a decreased aldosterone secretion.

Calcium exists as ionized, protein bound and complexed fractions. About 50% of plasma calcium is bound to the plasma proteins (Blaine, Chonchol, & Levi, 2014). The plasma calcium concentration of male rats at 12 month of age was significantly higher than that of 3, 6 and 9 month old rats. However, the plasma calcium of the female rats at 12 months was significantly higher when compared with rats aged 1 and 3 months but significantly lower than 9 month old rats. The reabsorption of calcium in the proximal convoluted tubule parallels that of sodium and water. Therefore, in conditions of extracellular volume contraction, plasma calcium concentration rises because of increased proximal tubular sodium reabsorption (Guyton & Hall, 2010).

Another factor that influences calcium reabsorption is the plasma concentration of phosphate. An increase in plasma phosphate stimulates parathyroid hormone, which increases calcium reabsorption by the renal tubules (Sulemanji & Vakili, 2013). The plasma phosphate concentration of male rats at 12 month of age was significantly higher when compared with other age. This could also explain the increase calcium concentration that was observed in the plasma of this group. Similarly, the urine calcium concentration of 12 month old rats were significantly higher when compared with rats of other age group. A strong positive correlation existed between the urine protein and urine calcium of the 12 month old female rats, although no linear correlation was found between the urine protein and urine calcium of the male rats. The increase in urine concentration of calcium in these groups may be related to their significantly higher urine protein. Calcium can be bound to albumin and globulins. For each 1.0 g/dL decrease in serum albumin, total serum calcium decreases by 0.8 mg/dL. For each 1.0 g/dL decrease in serum globulin fraction, total serum calcium decreases by 0.12 mg/dL (Friedman & Gesek, 1995). Hence, the loss of high amount of calcium in the urine may have resulted from greater urine protein excretion.

The kidneys regulate the phosphate concentration in the extracellular fluid by altering the rate of phosphate excretion in accordance with the plasma phosphate concentration and the rate of phosphate filtration by the kidneys. The urine phosphate concentration of the 12 month old rats was significantly higher when compared with rats of other age groups. An increase in plasma phosphate stimulates parathyroid hormone secretion. Parathyroid hormone decreases the transport maximum for phosphate by the renal tubules, so that a greater proportion of the tubular phosphate is lost in the urine (Sulemanji & Vakili, 2013). Thus, whenever plasma parathyroid hormone is increased, tubular phosphate reabsorption is decreased, and more phosphate is excreted.

Plasma EPO was reported to be higher in the aged rats than in younger rats (Kario, Matsuo, Kodama, Nakao, & Asada, 1992). Also, there are reports that the lifespan of red blood cells (RBCs) is shorter in older adults, and as a result, the percentage of red blood cells released from the bone marrow increases progressively with age (Lurie et al., 2005; Shperling & Danon, 1990). The red blood cell count and hematocrit of the 9 month old rats were significantly higher than that of other age group while that of 12 month of age was significantly lower when compared with rats of other ages. The high RBC counts in 9 month old rats may have resulted from increased erythropoietin secretion, which stimulates the bone marrow to increase red blood cell production. However, the decreased RBC counts and hematocrit in 12 month old rats when compared with other age group was probably due to the decreased response of bone marrow to EPO in rats (Koury & Bondurant, 1991) or increased destruction of the RBC in peripheral circulation. However, this requires further investigation.

The variations in most RBC parameters between neonatal and adult RBC are more pronounced in rats than in humans. Thus, the mean RBC volume was about 2.5 times more in neonatal than in adult rats, but about 1.2 times greater for human RBCs (Matovcik et al., 1986). The mean corpuscular volume of rats aged 1 and 3 months was significantly higher when compared with 6, 9 and 12 month old rats. This is probably owing to sequestration of macrocytic neonatal and young red blood cells and incomplete replacement of fetal red cells by smaller, adult-type cells (Endgstrom & Ohlsson, 1990).

Cell shrinkage through potassium and water loss has been documented under a variety of pathological circumstances, and appears regularly to precede spherocyte formation (Michael, 2014). The mean corpuscular hemoglobin concentration (MCHC) of rats aged 6, 9 and 12 months was significantly higher when compared with 1 and 3 month old rats. Also, rats aged 12 months had MCHC that was significantly higher than that of 6 and 9 months old rats. The increase in MCHC could be attributed to enhanced water and electrolyte loss from the cells, or loss of membrane not affecting the cell configuration (Ganzoni, Barras, & Marti, 1976). This assertion is substantiated by the increase in urine potassium concentration that was recorded in the 12 month old rats.

The white blood cell, granulocyte and monocyte counts of the 12 month old rats were lower when compared with rats of other ages. This shows that the rats may be under stress resulting from an increased metabolic need for replacement of aging cells which resulted in the release of epinephrine. The initial response to epinephrine release begins in the amygdala, which triggers a neural response in the hypothalamus. This is followed by activation of the pituitary gland and secretion of the adrenocorticotropic hormone (ACTH) (Margioris & Tsatsanis, 2013). The adrenal gland is activated almost simultaneously and releases epinephrine. The release of chemical messengers results in the production of the cortisol, which increases blood pressure, blood glucose, and suppresses the immune system (Padgett & Glaser, 2003).

5. Conclusion

Wistar rats both sexes excreted considerable amounts of protein in their urine with the older rats having higher urine protein than the younger ones. Increased urine sodium and potassium indicated that either the changes in the proximal tubule are of magnitude to impair tubular reabsorption of sodium or decreased absorption of sodium occurs in the more distal tubular segments. The 3 months old rats had reduced and consistent plasma and urine concentrations of electrolytes measured when compared with rats of other age groups. This suggests that they are least affected by proteinuria, which implies that long-term renal studies involving the use of rats must be carefully interpreted because of the changes in plasma and urine concentrations of electrolytes as the rats age.

The results of this study may stimulate further mechanistic research on the effects of urinary protein excretion on renal handling of electrolytes in rats.

Competing interest

The authors declare no conflict of interest for this study.

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