The Haryana Veterinarian (June, 2025) **64(1),** 77-80 ISSN 0033-4359 (P) **Research Article**

A PRELIMINARY INVESTIGATION TO ASSESS THE AGE-RELATED RENAL MANIFESTATIONS IN THE SERUM AND URINE OF MALE WISTAR RATS

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ABSTRACT

Kidneys are more prone to aging and age-associated renal problem, it is a tough challenge in clinical setup. Animal models are indispensable for understanding the pathophysiology of kidney diseases and for pre-clinically testing novel remedies. This prompted us to assess the presence of the age-related kidney biochemical changes in 15 months old Wistar male rats prior to pre-clinical therapeutic trial. Serum and urine of young (2 months) and old (15 months) Wistar male rats were collected following routine procedure. Creatinine and uric acid levels in serum and proteinuria were determined using biochemical assays. Old rats manifested elevated levels of above markers indicating the presence of age-related glomerular filtration changes. These findings suggest that Wistar male rats of age 15 month or more could act as potential laboratory animals for investigating age-related nephropathy.

Keywords: Age-linked kidney changes, Preliminary trial, Proteinuria, Rats

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The aging is a multifaceted biological phenomenon, which is marked by the loss of adaptive responses to physiological stress, resulting in an increased vulnerability to disease and death. With age, some of the mechanisms required to maintain organ function are impaired, which results in decreased repair capacity. Aging, as well as kidney disease in the older patients, is on the rise. Both conditions constitute a burden on the health care system (Abdel-Rahman and Okusa, 2014). Kidney diseases are more prevalent in dogs (Sidhu *et al.*, 2023) and cats. It is observed that 80% population of aged cats suffer from kidney problems (De Santis *et al.*, 2022). Kidneys are one of the most afflicted organs due to process of aging. Progressive loss in renal structure and function is one of the striking characters of aging kidneys. Ageing is relatively a speedy phenomenon in kidneys than other organs. Further, functional insufficiency in kidney put load on other organs and may contribute to their functional detrition (Lee *et al.*, 2018). Therefore, decelerating the biological processes involved in the ageing of the kidney could offer beneficial therapeutic approach for age-related nephropathy.

Animal models are indispensable for gaining insight about the pathophysiology of several kidney disorders and for pre-clinically testing novel agents. Rodents remain as frequently used models for mimicking clinical kidney diseases (Liang and Liu, 2023). With aging, the kidney of the laboratory rat also undergoes functional and structural changes (Haley and Bulger, 1983). Among sex, male rats have more predilections for aging where almost 80% of the population are affected by the second and final year of life (Owen and Heywood, 1986). The beneficial effects of a plant-derived compound were reported in our previous studies in experimentally-induced kidney injury models (Lingaraju *et al.*, 2015; Sharma *et al.*, 2017). Aging kidneys are good candidates for such an intervention, and the aging process in kidneys could be preventable (Kanasaki *et al.*, 2012). Therefore, anterior to therapeutic intervention studies we aspired to determine the presence of the age-related kidney biochemical changes in 15 months old Wistar male rats to assess the feasibility of their inclusion in to trial.

MATERIALS AND METHODS

Drugs and chemicals

Ketamine (NP8077A, Miracalus Pharma Ltd., India), xylazine (FHK8001, Indian Immunologicals Ltd., India), creatinine kit (1101060035, Coral Clinical Systems, India), uric acid kit (1102260075, Coral Clinical Systems, India), albumin kit (1101021150, Coral Clinical Systems, India), Bradford reagent (19219, Sisco Research Laboratories, India), bovine serum albumin (BSA) (044004, Central Drug House, India), sodium chloride (1932060521, Merck, India), protein loading buffer (ML096-5ML, HiMedia, India), sodium dodecyl sulphate (SDS) (194831, MP Biomedicals, US), TEMED (M146-50 ML, Amresco, US), ammonium persulfate (M133-100G, VWR, US), glycine (M103-1KG, Amresco, USA),

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pre-stained protein ladder (MBT092-100LN, HiMedia, India), TRIS (0497A, Amresco, USA), hydrochloric acid (HCl; 0017200500, Lobachemie, India), Acrylamide/ Bisacrylamide solution (786-505, G-Biosciences, USA), coomassie brilliant blue (CBB) (M140-25G, Amresco, USA), sodium chloride (1932060521, Merck, India), methanol (1945162521, Merck, India), acetic acid (60006325001730, Merck, India).

Animal experiments

Young (2 months) and old (15 months) male Wistar rats were procured from Laboratory Animal Resource Section of Indian Veterinary Research Institute (IVRI), Izatnagar. Animals were kept in cages and maintained ambient conditions (temperature of 23 ± 2º C, 40-60% relative humidity). They were given free access to drinkable water and laboratory animal feed. They were housed for15 more days in these managemental conditions for acclimatization. They grouped into two groups as young and old with 5 animals in each. Animals were weighed initially and were kept for 24 hours in metabolic cage for collection of urine. Urine samples were centrifuged at 2000 rpm for 10 minutes and sediment was discarded to collect clear urine supernatant. The blood samples (4 ml) were collected from the retro-orbital plexus of anesthetized animals using ketamine (50 mg/kg BW, i/p) and xylazine (5 mg/kg BW, i/p). 3 ml of blood was allowed to coagulate for 2 hours and serum was then separated by centrifuging the blood collected tubes at 2000 rpm for 10 minutes. The collected serum and urine was stored at -20º C for later biochemical analysis. Experiments on rats were conducted with due approval of Institutional Animal Ethics Committee of ICAR-IVRI, Izatnagar.

Serum and urine analysis

Creatinine in serum was estimated using commercially available kit as per the protocol mentioned in the user manual. Briefly, 30 µl of serum is mixed with 300 µl of picric acid reagent and centrifuged at 3000 rpm for 10 minutes to obtain clear supernatant. From this, 165 µl samples supernatant was mixed and incubated with 15 µl of alkaline buffer reagent for 20 minutes. Additionally, 15 µl of creatinine standard or blank (distilled water) were incubated with 150 µl of picric acid reagent and 15 µl alkaline buffer reagent for 20 minutes. The absorbance of the incubated mixture was then read at 520 nm (BioSpectrometer® Basic, Eppendorf, Germany). The absorbance of known concentration of creatinine standard was used to estimate the amount of creatinine in test samples.

Uric acid in serum was estimated using commercially procured kit and following the instructions provided in the user manual. Serum samples or uric acid standard were incubated with working reagent containing uricase enzyme, 4-aminoantipyrine and phenolic compound for 5 minutes. The absorbance of the resultant red coloured quinoneimine dye complex was then read at 520 nm (BioSpectrometer® Basic, Eppendorf, Germany). The absorbance of known concentration of uric acid standard was used to estimate the amount of uric acid in test samples.

Total protein concentrations in urine were estimated by Bradford method as described previously with little modifications (Bradford, 1976). 5 µl of urine supernatants or BSA standard were incubated with 245 µl of Bradford reagent for 5 minutes at room temperature and the OD of the mixture was read at 595 nm (BioSpectrometer® Basic, Eppendorf, Germany). The amounts of total protein in samples were estimated from standard curve constructed using known concentration of BSA ranging from 0-100 µg/ml.

Urine albumin levels were estimated using a commercial kit. 10 µl of urine samples or albumin standard were mixed with 1 ml of bromocresol green (BCG) reagent and incubated for 5 minutes at room temperature. The absorbance of the resulting product was read at 630 nm (BioSpectrometer® Basic, Eppendorf, Germany).The absorbance of known concentration of albumin standard was used to estimate the amount of albumin in test samples.

SDS polyacrylamide gel electrophoresis (PAGE) was conducted to analyse the protein pattern of urine samples. Glass plates with the 1 mm spacers were fitted onto casting apparatus and 10% resolving gel (pH 8.8) was prepared and poured into the space between the plates and kept for 20 minutes for polymerization. Later, 4% stacking gel (pH 6.8) was prepared and poured over resolving gel and immediately comb was inserted. This was kept for 15 minutes for polymerization. Glass plates containing gel was shifted to electrode assembly/running module and filled with SDS-PAGE running buffer (pH 8.3). Around 10 µg of urine total protein was mixed with 5X protein loading buffer and boiled for 5 minutes in water bath. These samples were carefully loaded into wells of SDS-PAGE gel along with a commercial pre-stained protein ladder. Thereafter, the electrophoresis was conducted at 90 V for 50 minutes. After the completion of electrophoresis, the gel was subjected for staining using CBB and de-stained using aqueous mixture of methanol and acetic acid to analyse protein pattern.

Statistical analysis

GraphPad Prism-8 software was used for statistical analysis. Data were analysed by unpaired t-test and expressed as mean±standard error of mean (S.E.M). p value less than 0.05 was assumed as statistically significant.

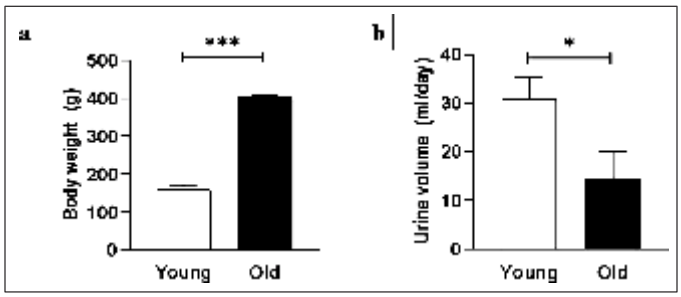

Fig. 1. Body weight of rats from young and old group (a) Twenty four hour urine volume voided by rats from young and old group (b) *data are statistically significant between the groups (* p<0.05; ***p<0.001).

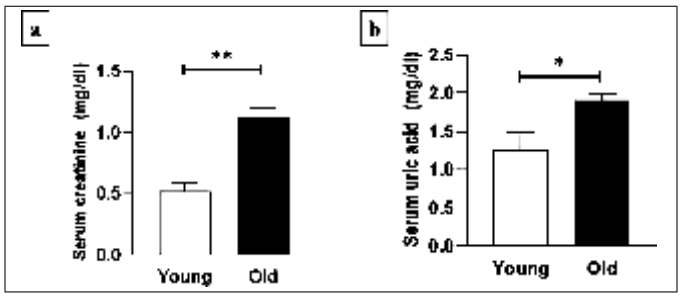

Fig. 2. Serum creatinine (a) and serum uric acid (b) in rats from young and old group. *data are statistically significant between the groups (* p<0.05; **p<0.01).

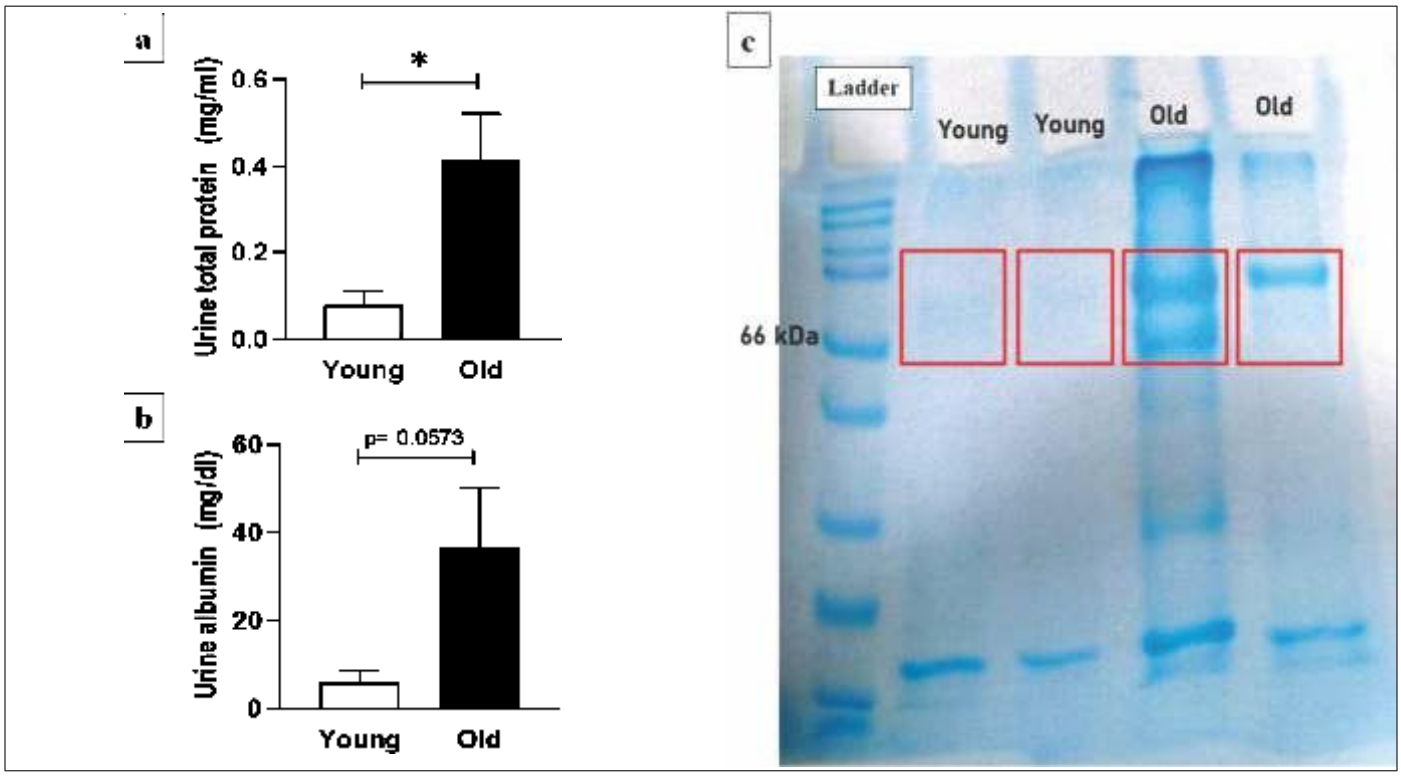

Fig. 3. Urine total protein (a), urine albumin (b) and representative photograph of SDS-PAGE of urine samples (c) of rats from young and old group. *data are statistically significant between the groups (* p<0.05).

RESULTS AND DISCUSSION

Serum and urine markers of renal function in young and old rats

Not to mention in particular, the body weight of young rats were lesser compared to old rats. But, conversely the volume of urine output per day was significantly lesser in old rats than the less weighing young rats revealing the reduced level of glomerular filtration (Fig. 1). Serum creatinine and uric acid level were considerably more in old rats indicating disturbances in renal clearance whereas they were markedly low in young rats (Fig. 2).

As age advances the kidney manifests depletion in glomerular filtration with loss of functional nephrons. There is a reduction in amount of renal parenchyma and total kidney volume reduces by about 16 cm^3 per decade and this decline is more distinct after the age of 60 years (Dybiec *et al.*, 2022). With aging, many studies revealed gradual decline in glomerular filtration rate (GFR) and renal blood flow due to an increase in renal vasculature resistance (Czarkowska-P[1]czek *et al.*, 2018). Decreased GFR results in an increase in serum creatinine and uric acid levels (Glassock and Winearls, 2009; Wang *et al.*, 2021).

Further, in this study along with reduced GFR as discussed above the filtration barrier was found to be disrupted in old rats as reflected by enhanced total protein, albumin and other high molecular weight (HMW) protein excretion in the urine which was not present in the young rats. SDS-PAGE images showed a visible rise in the urinary HMW proteins in old rats (Fig. 3).

Proteinuria, particularly excretion of albumin is a

frequent feature in age-related renal dysfunction and, by large, correspond to intensity of damage to glomerulus. Excretion of total protein becomes ten times more in aged rats due to abnormal passage of HMW plasma proteins through filtration barrier (Alt *et al.*, 1980; Goldstein *et al.*, 1980).

To summarize, serum and urine profile of 15 months old Wistar male rats were distinctively apart from 2 months old young Wistar male rats to cover essential aspects of renal aging. Aged rats embody a great potential for investigating pathological mechanisms and testing the therapeutic effect of compounds in age-related nephropathy.

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