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Kidney damage induced by sub-chronic fine particulate matter exposure

Iván Tavera Busso[a,b,*], Ana Carolina Mateos[a], Luis Isaías Juncos[b], Norma Canals[c], Hebe Alejandra Carreras[a]

[a] Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET, Departamento de Química, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba, Argentina
[b] Fundación J. Robert Cade, Córdoba, Argentina
[c] Instituto Oulton, Córdoba, Argentina

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ABSTRACT

According to the WHO, about 3 million people die each year due to ambient air pollution. Most of the *in vivo* studies on the $PM_{2.5}$ effects have been done on respiratory and cardiovascular tissues. However, little is known about the effects on the tissues involved on xenobiotic removal, such as kidneys. In the present study we assess the harmful effects of sub-chronic exposure to $PM_{2.5}$ on the kidney, by investigating histologic and serum alterations in healthy and hypertensive rat models. Mean $PM_{2.5}$ concentrations during exposures were slightly above the daily WHO standard. Exposed animals showed fibrosis, mesangial expansion, decrease glomerular and tubular lumen volumes in kidneys, with an elevated BUN. Hypertensive animals also exhibited much more severe alterations than healthy animals. We conclude that $PM_{2.5}$ induces minimal or small-scale abnormalities that can be determinant for renal health preservation.

1. Introduction

Epidemiological evidences on air pollution health effects have significantly increased in recent years and have enhanced the notion that it may embody a major environmental risk factor. Indeed, atmospheric particulate matter (PM) having a diameter equal or < 2.5 μm ($PM_{2.5}$) seem to be a reliable indicator to estimate air pollution impact (World Health Organization, 2016), as exposure to small particles has negative health effects even at low concentrations and below the standards suggested by the World Health Organization (Janssen et al., 2011; Riva et al., 2011; Busso et al., 2017).Actually, close to 3 million people die every year due to air pollution related diseases, representing about 16% of the deaths due to non-communicable diseases (World Health Organization, 2016). Furthermore, most of this mortality is linked to cardiovascular complications (Mills et al., 2009). Particularly, several epidemiological (Wellenius et al., 2006; Harrabi et al., 2006; Auchincloss et al., 2008; Franck et al., 2011; Ying et al., 2014) and experimental studies (Brook, 2007; Franklin et al., 2015) have shown a positive correlation between blood pressure and $PM_{2.5}$ exposure, most likely due to vasoconstrictor effects induced by these particles (Mills et al., 2007; Franklin et al., 2008; Aragon et al., 2016). This seems exceedingly important given the toxic effects of $PM_{2.5}$ and the fact that it is estimated that by the year 2020, noncommunicable diseases could account for 60% of the global disease burden, causing a 73% of the deaths (World Health Organization, 2014).

Most of the *in vivo* studies on the $PM_{2.5}$ effects have been done on respiratory (Seagrave et al., 2006; Bonner, 2007; Reed et al., 2008) and cardiovascular tissues (Sun et al., 2005; Wang et al., 2015). In fact, although it is easy to envisage $PM_{2.5}$ gaining access into the respiratory system and translocating into the circulation (Polichetti et al., 2009), the toxicokinetic mechanisms are not fully elucidated and little is known about the $PM_{2.5}$ effects on the tissues involved on xenobiotic removal, such as liver and kidneys. Recently, Miller et al. (2017) have shown that inhaled particles can not only cross the alveolar-capillary barrier thus reaching remote tissues but can also be excreted in the urine. Nevertheless, the relationship between $PM_{2.5}$ chronic exposure and changes in renal function are still poorly understood (Seltenrich, 2016).

Up until now, few epidemiological studies have shown firm evidences for early renal disfunction following particulate matter exposure (Mehta et al., 2016; Bowe et al., 2017; Kim, 2017). Also, current literature shows rather few reports on kidney histologic alterations during particulate matter exposure (Damek-Poprawa and Sawicka-Kapusta, 2003; Aztatzi-Aguilar et al., 2016). Moreover, they do not address human actual exposure to urban atmospheric pollutants, nor the particles natural route of access into the body. Thus, in the present study

* Corresponding author at: Av. Vélez Sarsfield 1611, X5016 GCA Córdoba, Argentina.
E-mail address: ivantaverabusso@outlook.com (I. Tavera Busso).

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our aim was to assess the harmful effects of sub-chronic exposure to $PM_{2.5}$ on the kidney. For this, we investigated both histologic parameters and serum chemical alterations. Moreover, and taking into account that hypertension is a key world's morbidity cause (World Health Organization, 2013), we also evaluated the $PM_{2.5}$ effects in the Spontaneously Hypertensive Rat model (Kodavanti et al., 2000).

2. Materials and methods

2.1. Sampling site and study design

Sampling site and study design has been previously described by Busso et al. (2017). Briefly, daily $PM_{2.5}$ samples were collected at the roof of the Chemistry Department of the Faculty of Exact, Physical and Natural Sciences (National University of Córdoba, 31°26′11.429″S; 64°11′38.191″W), at 7 m from the ground. At the same place, healthy and spontaneously hypertensive rats were exposed in a mobile animal facility to urban air. After the exposure period, serologic parameters were measured, and the kidneys were removed for histologic examination.

2.2. Exposure protocol

The protocol was completed complying with the Institutional Animal Care and Use Committee guidelines of the USA National Institute of Health. Six 5-week old male Wistar-Kyoto rats (W-K) and six 5-week-old male Spontaneously Hypertensive Rats (SHR) were allocated in an air-filtered bioresources environment at the "J. Robert Cade" Foundation before starting the experiments. The animals in both strains were randomly divided in two equal groups (control and treated) and held in one of the mobile animal facility chambers for a period of three months (2160 h) (Barile, 2013). Each chamber air uptake was connected to a Harvard Impactor (HI). Thus, in the control chamber all particles were removed with an impaction plate and a $PM_{2.5}$ polytetrafluoroethylene (PTFE) filter; whereas in the treatment chamber only large particles ($> PM_{2.5}$) were removed by the impaction plate (no filter was used). A 12.5 L min^{-1} airflow was employed to ensure no hypoxia in exposed animals and a full replacement of the internal atmosphere at least 15 times per hour.

The experimental groups were design as follow: W-K Control; SHR Control; W-K Treatment and SHR Treatment. Water and food were supplied *ad libitum* and beds were renewed twice a week with sieved sawdust. This exposure protocol was repeated three times employing new animals, during the cold seasons (from May to October), in the constructed 2015 and 2016.

2.3. $PM_{2.5}$ sampling and mass determination

Daily $PM_{2.5}$ samples were obtained with an HI coupled to the control chamber in 47-mm PTFE filters with a 2.0 μm pore (*Zefluor*, *Millipore*). Since air flow was lower than that suggested by the HI manufacturer, a cut point slightly over 2.5 μm was expected. The mass of collected particles was verified by gravimetric differences using a microbalance (0.01 mg mass resolution, *Sartorius*) (Busso et al., 2017). Results were expressed as mean ± standard error (SE).

2.4. Blood and tissue collection

To obtain blood and tissue samples, all rats were weighed and then anesthetized using a mixture of xylazine (20 mg/kg^{-1} body weight) and ketamine (100 mg/kg^{-1} body weight) (Busso et al., 2017). Then, 5-mL blood samples were drawn by cardiac puncture for serologic measurements. The kidneys were excised at the hilum and the fasciae removed. In all rats, both kidneys were weighed to estimate the somatic index and then, they were dissected longitudinally through the hilum, washed with cold phosphate buffered saline solution (PBS, 4 °C) and gently compressed to remove excess blood. One fragment was weighed (wet weight, WW) on an analytical balance (resolution 0.1 mg) and dried at 60 °C up to constant weight (DW) for elemental composition measurements. A second fragment was placed in 100 mL of formaldehyde neutral buffer solution for histological assessment (Bancroft et al., 2013).

2.5. Elemental composition

The levels of B, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Ba, Tl, Pb and Bi were measured in filters with fine particles and renal tissues by Mass Spectroscopy Inductively Coupled Plasma (*Agilent 7500cx*) as previously reported by Busso et al. (2017). High, middle and low concentrations calibration curves were made employing pure standards for all elements (*Sigma*).

2.6. Tissue analysis

The formaldehyde fixed kidneys were dehydrated with ethanol solution, clarified by xylol and embedded in paraffin by an automated tissue processor (*Leica TP1020*). Fragments were then cut with a rotary microtome (2 μm) and mounted on slides. In each rat six tissue slides (3 from each kidney) were obtained. These slides were deparaffinized and stained as follows: one sample with hematoxylin/eosin (HE), a second sample with periodic acid-Schiff (PAS, 2) and a third sample with Masson trichrome (MTS) (Bancroft et al., 2013). 15 fields in each slide were examined at 100, 400 and 1000× (*Olympus CX31*) searching for leucocyte infiltration, fibrosis, mesangial expansion, anisokaryosis, anisocytosis and any other pathologic sign (Kumar et al., 2017; Aztatzi-Aguilar et al., 2016). Each field was analyzed and scored using a scale from 0 to 3 to rank the damage (0: minimum or no observed, 1: mild, 2: moderate and 3: severe) and the slide mean was calculated. Percent of glomerular and tubular lumen (100×, %) and glomerular size (400×, pixels) were also determined in 20 field photographs per animal (400×) by a computer image analysis software (*Bio7*, Freeware). Results of each group were expressed as mean ± SE.

2.7. Serum parameters

Glycemia (enzymatic glycemia AA, *Wiener lab.*), blood urea nitrogen (BUN, enzymatic urea, *Wiener lab.*), uric acid (Uricostat enzymatic AA, *Wiener lab.*), creatinine (AA kinetic creatinine, *Wiener lab.*) and proteinogram (electrophoresis) were measured in the serum samples collected from each animal (Burtis and Bruns, 2007).

2.8. Statistical analysis

Total inhaled mass (TIM, μg) during the 90 days exposure period was calculated summing up daily inhaled mases for each animal. They were calculated multiplying $PM_{2.5}$ concentration (μg m^{-3}) by the maximum ventilation rate (m^3 min^{-1}) and the exposure minutes per day (min) (Sharp and Villano, 2012).

Differences in $PM_{2.5}$ concentration, TIM, and particles elemental composition between expositions were evaluated by ANOVA with LSD Fisher. Student's *t*-test was used to assess statistical differences between treatments (considering strains as independent blocks) and strains (considering treatments as independent blocks). Differences with a p value < 0.05 were considered statistically significant (*IBM SPSS 19.0*, IBM Corp.). In addition, Pearson coefficients were calculated to assess associations between air and tissues elemental composition, as well as between histological and serum parameters.

3. Results and discussion

$PM_{2.5}$ concentrations and elemental compositions for each exposure are shown in Table 1. Overall daily $PM_{2.5}$ mean was

Table 1
$PM_{2.5}$ daily concentration (μg m^{-3}), TIM (μg) and elemental composition (ng m^{-3}) for each exposition period.

Determination	Period 1 (05/2015–08/2015)	Period 2 (08/2015–11/2015)	Period 3 (05/2016–08/2016)
$PM_{2.5}$ (mean)	29.6 ± 4.8	30.4 ± 5.3	28.1 ± 4.7
$PM_{2.5}$ (median)	8.2	18.9	13.2
$PM_{2.5}$ (minimum)	1.0	9.5	2.6
$PM_{2.5}$ (maximum)	114.0	73.0	66.4
TIM	366.9	314.2	373.1
B	4.00 ± 0.36	2.59 ± 0.31	5.40 ± 0.72
V	0.16 ± 0.06	0.58 ± 0.06	1.02 ± 0.06
Cr	0.80 ± 0.16	1.77 ± 0.18	3.32 ± 0.16
Mn	2.57 ± 0.81	8.96 ± 0.89	13.78 ± 0.79
Fe	105.09 ± 38.89	404.95 ± 42.6	721.33 ± 37.57
Co	< DL	< DL	< DL
Ni	< DL	1.44 ± 0.43	2.02 ± 0.27
Cu	1.44 ± 2.32	4.74 ± 2.55	17.7 ± 2.43
Zn	114.97 ± 16.2	359.40 ± 17.75	55.44 ± 17.15
As	0.13 ± 0.04	0.17 ± 0.03	0.39 ± 0.03
Sr	0.85 ± 0.23	0.58 ± 0.2	3.11 ± 0.18
Mo	0.27 ± 3.65	0.32 ± 3.11	12.52 ± 3.37
Cd	0.03 ± 0.01	0.04 ± 0.02	0.19 ± 0.02
Ba	2.29 ± 0.77	6.04 ± 0.84	14.03 ± 0.8
Tl	0.07 ± 0.03	0.53 ± 0.05	0.21 ± 0.03
Pb	1.52 ± 0.58	1.88 ± 0.55	6.18 ± 0.51
Bi	0.02 ± 0.005	0.03 ± 0.003	0.01 ± 0.005

Ref.: " < DL", below detection limits.

29.4 ± 2.8 μg m^{-3}, which is slightly above the daily WHO standard (25 μg m^{-3}) (World Health Organization, 2016). Even more, the daily $PM_{2.5}$ levels exceeded this standard value 14 to 24 days out of the 90 exposure days. This is attributed to the frequent thermal inversion events, a feature of the city of Córdoba during wintertime (Olcese and Toselli, 2002). The maximum values registered at each exposition were associated with forest fires taking place at hills near the city (Achad et al., 2014). Results from different periods were analyzed all together, as TIM and $PM_{2.5}$ mean concentration did not show significant differences between the sampling exposures ($p > 0.05$ for both parameters).

Our results are in agreement with those reported for the same city by Lanzaco et al. (2017) in similar periods. Nevertheless, the concentration levels of particles were lower than those measured in 2014 (Busso et al., 2016), which may be explained by the high humidity registered during that period.

Regarding the elemental composition, Zn and Fe were the most abundant, whereas the levels of the remaining elements were two orders of magnitude lower, in agreement with the results reported by Lanzaco et al. (2017). Indeed, we found levels of Mn, Cu, Sr and Ba similar to those reported by Lanzaco et al., whereas concentrations of V, Cr, Ni, As and Pb were slightly lower. The exception was Mo levels, which were remarkably higher in our study in period 3. Although the concentrations of B, Mn, As, Sr, Cd, Tl, Pb and Bi were found within levels regarded natural, some elements such as Tl, Pb and Bi showed maximum values exceeding the reported levels of natural environmental concentrations (Kabata-Pendias and Mukherjee, 2007; Kot, 2009; Rogula-Kozłowska et al., 2015). Cr, Fe, Ni, Zn and Ba showed mean concentrations far above natural levels, suggesting that majority of $PM_{2.5}$ has mainly an anthropic origin.

Regarding exposure characterization, the maximum and minimum mean temperature inside the chambers were 26.4 ± 1.7 °C and 19.1 ± 1.1 °C, respectively. Minimum and maximum temperatures registered in all outside chambers periods were 2.6 °C and 24.1 °C. These are values in which thermal inversion phenomena tend to occur (Olcese and Toselli, 2002).

Tissue inorganic compositions were also analyzed searching for associations with air concentrations (Fig. 1) and with histologic alterations. Fe was excluded from this analysis since it is a major component of blood. Co is not reported because their levels were below detection limits. Tissues from W-K animals showed higher levels of all elements than SHR, except for Mn, Mo and Cd. In both groups, a linear pattern was observed for all the measured elements between tissues and particles levels suggesting a clear association or dependence between tissues and air concentration. This hypothesis is also based on the findings of Damek-Poprawa and Sawicka-Kapusta (2003), who reported high Cd renal levels in rodents belonging to populations bordering steelworks and smelters. Similarly, Miller et al. (2017) showed that inhaled inert gold nanoparticles enter the bloodstream and are detected in the urine a few minutes after the exposure. Furthermore, Wallenborn et al. (2009) found that the instillation of ^{70}Zn rises endogenous zinc in the liver, implying that exposure to exogenous elements may modify normal hepatic metabolism.

Several somatic parameters were measured in kidney tissues to assess damage produced by $PM_{2.5}$ exposure in control and treated animals (Table 2). The proportion of tissue hydration did not show significant differences between treatments but did so between strains ($p > 0.05$). In fact, SHR showed a lower degree of tissue hydration. Somatic index values can be considered as normal (8–9 mg organ g^{-1} animal), although W-K animals showed statistical differences (Sharp and Villano, 2012). In contrast, SHR showed renal hypertrophy, although differences between controls and treatments did not reach statistical significance. Hypertrophy could be considered a sign of decreased functional capacity of the kidney, as it occurs in hypertension (glomerular hypertrophy) (Gómez Llambí and Piskorz, 2013). Thus, this effect may be related to the physiological status of SHR or exposure to substances not retained in filters. However, this hypertrophy may be related to microvasculature alterations, characteristically of hypertension, which makes the organ more susceptible to the action of harmful substances (Van Vleet and Schnellmann, 2003).

Regarding histological analysis (Table 2), animals from both strains exposed to $PM_{2.5}$ did not show anisocytosis or anisokaryosis. A slight leukocytes increase was observed in the renal interstitium in both strains, with a predominance of diffuse medullar infiltration and focal periglomerular infiltration (Fig. 2a, b). On the other hand, some SHR tissues presented fibrous patches (Fig. 3a, b, c) and thickening of tubular basement membranes (Fig. 4a, b).

Fibrosis is usually a response to a chronic inflammatory process and is described in most chronic kidney diseases (Mezzano and Aros, 2005), therefore SHR sensitivity to fibrosis may be related to its pathological condition. Thus, to protect glomerular capillaries from high blood

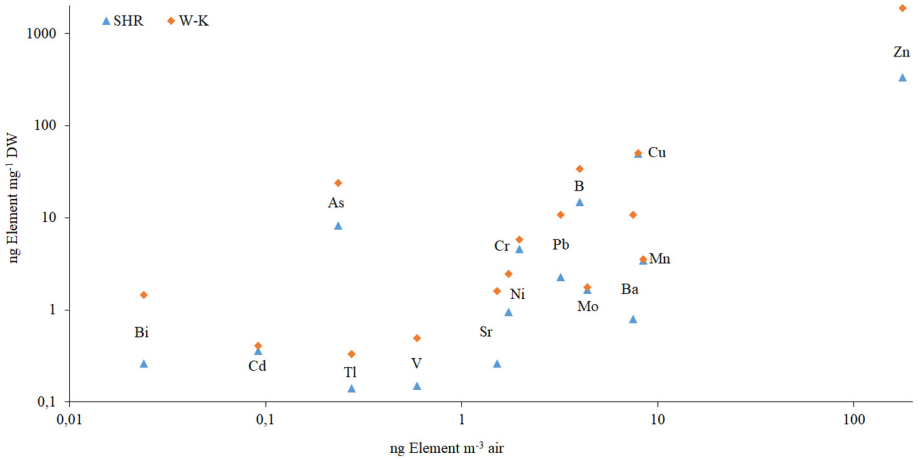

Fig. 1. Air elemental composition *vs.* tissue elemental composition.

pressure, a vasoconstriction of the afferent arterioles occurs, generating ischemia (Kumar et al., 2017). If this situation persists, ischemia may lead to cellular hypoxia and necrosis and thereby normal tissues may be replaced by fibrous tissues (Gómez Llambí and Piskorz, 2013). Although these events are already present in hypertensive animals, the exposure to $PM_{2.5}$ could enhance the damage. In fact, our results agree with Aztatzi-Aguilar et al. (2016), who reported damage in tubular structures and leukocytic infiltrate associated with a profibrogenic state in kidneys from healthy animals intermittently exposed to urban $PM_{2.5}$ ($23.5\ \mu g\ m^{-3}$).

Regarding glomerular changes, the SHR exposed to fine particles showed capillary congestion (Fig. 5a, b) and reduction of the glomerular urinary space which was associated to a mild mesangial expansion ($r = -0.49$) (Fig. 6a, b). Furthermore, this mesangial expansion showed a positive correlation with the degree of fibrosis ($r = 0.65$). These histological changes are frequent in hypertensive membranoproliferative glomerulonephritis (Wilcox and Tisher, 2008) and in diabetic nephropathy (Kanwar et al., 2008). On the other hand, we show no differences in glomerular lumen and mesangial expansion between control and treated W-K animals. Nevertheless, treated SHR presented a higher mesangial expansion rate than W-K ones, suggesting that hypertense individuals would be more susceptible to fine particles than healthy ones.

The mechanisms underlying mesangial expansion are not completely understood. It is hypothesized that it results from protein accumulation in the mesangial extracellular matrix due to insufficient degradation and/or an impaired repairing process involving the thickening of the glomerular basement membrane (Wilcox and Tisher, 2008; Kumar et al., 2017). In such a case, damage would be mediated by fibronectin, a protein that stimulates the production of collagen, leading to progressive sclerosis. Thus, exposure to $PM_{2.5}$ could activate repairing mechanisms in kidneys (Aztatzi-Aguilar et al., 2016) inducing the expression of fibronectin (Park et al., 2008; Krimmer et al., 2013).

In addition to tissue fibrosis, tubules in both strains exhibited regenerative lesions and reduction of the urinary space as compared to control groups. Also, a marked eosinophilic infiltration and detachment of epithelial cells (Fig. 7), both common processes in hypoxia (Gerosa et al., 2015) were observed.

Regarding serum parameters (Table 3), no statistical differences were found in glycemia, uric acid, creatinine or blood proteins levels (total and fractions) between treatment groups. However, a significant increase in BUN was observed in animals of both strains exposed to $PM_{2.5}$ (Kurtz and Travlos, 2017). These results are consistent with those previously reported by Riediker et al. (2004), who found a positive relationship between suspended traffic particulate matter and increased BUN levels, several hours after the inflammatory stimulus (Riediker, 2007). Furthermore, it is known that the BUN tends to increase in toxic situations, dehydration, liver disorders and protein catabolism (Kumar et al., 2017), all of which is consistent with the general condition of some exposed animals. These facts may explain why creatinine levels were normal, while the BUN was slightly above the normal levels and hence the altered BUN:creatinine relationship. BUN showed significant correlations with glomerular lumen in both strains ($r = -0.59$, W-K; $r = -0.54$, SHR). BUN also showed significant correlations with the infiltrate degree ($r = 0.61$) and tubular lumen ($r = -0.67$) in W-K animals; and mesangial expansion ($r = 0.53$) in SHR. This suggests a relationship between the altered renal function and the tissue changes (Balakumar et al., 2008).

These general effects detected in exposed animals, frequently more severe in SHR, may be due to a pulmonary inflammatory response that leads to systemic inflammation, pollutants that induce a disturbance in the autonomic nervous system affecting blood pressure, or to the direct

Table 2
Somatic and tissue determinations en WK and SHR animals (mean ± SE).

Parameter	W-K			SHR		
	Control	Treatment		Control	Treatment	
Somatic index (mg organ g^{-1} animal)	8.82 ± 0.67	6.41 ± 0.58	*	11.0 ± 0.81	10.8 ± 0.76	
Tissue hydration (%)	80.7 ± 0.34	80.2 ± 0.29		77.8 ± 4.16	79.9 ± 3.89	
Infiltration (score)	0.01 ± 0.01	0.20 ± 0.01	*	0.07 ± 0.02	0.20 ± 0.02	
Fibrosis (score)	0.03 ± 0.03	0.1 ± 0.02		Not Observed	0.22 ± 0	*
Glomerular lumen (%)	40.2 ± 2.49	38.6 ± 2.16		45.4 ± 1.57	38.7 ± 1.47	**
Glomerular size (pixels)	1410 ± 69	1400 ± 60		1449 ± 65	1480 ± 61	
Mesangial expansion (score)	0.40 ± 0.10	0.45 ± 0.09		0.38 ± 0.12	1.15 ± 0.12	**
Tubular lumen (%)	49.7 ± 1.70	44.4 ± 1.47		42.3 ± 0.72	35.4 ± 0.67	***

Ref.: "*", $p < 0.05$; "**", $p < 0.01$; "***", $p < 0.001$.

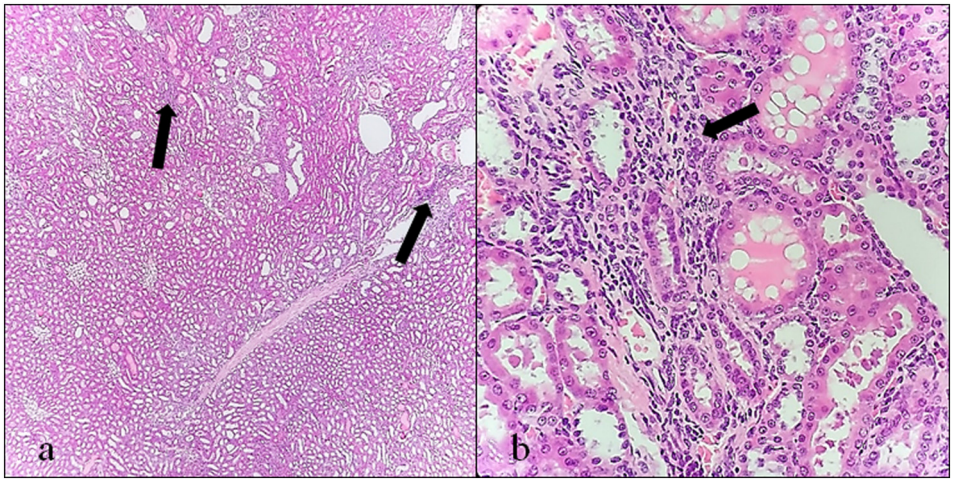

Fig. 2. a, b - Leukocytic infiltrate (HE) at 100× (2a) and 400× (2b) in an exposed W-K animal. Arrows show pericapsular foci f leucocytes in the renal cortex zone.

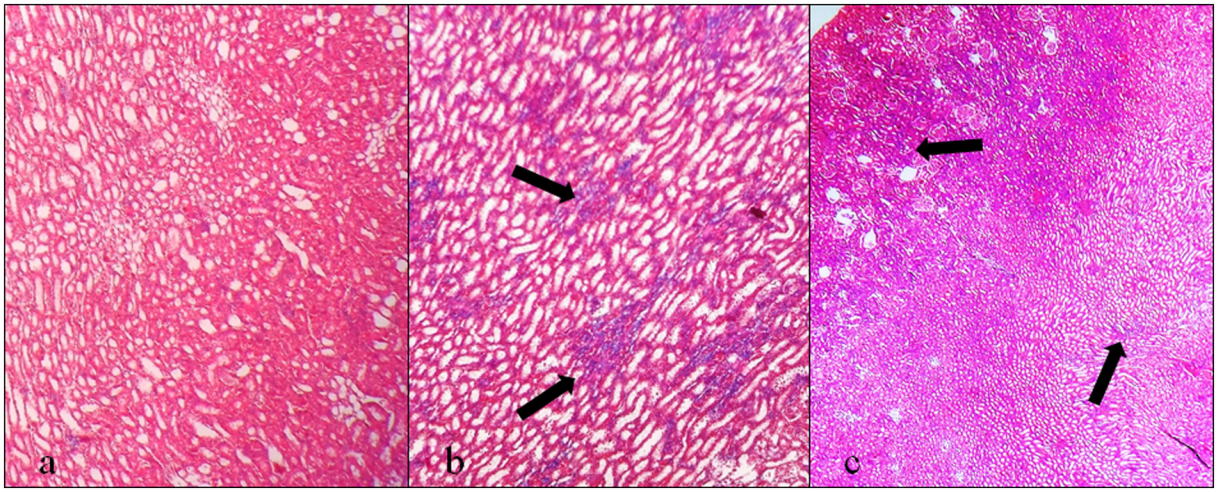

Fig. 3. a, b, c - Renal fibrosis (MTS) in a control (3a, 400×) and exposed (3b, 400×; 3c, 100×) SHR. Arrows indicate foci of fibers.

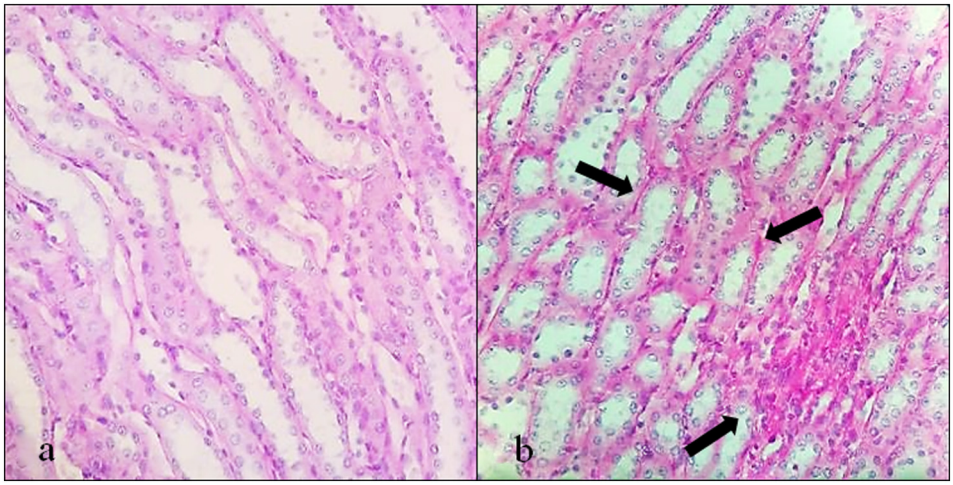

Fig. 4. a, b - Thickening of tubular basement membrane (PAS, 400×) in control (4a) and exposed (4b) SHR.

action of particles on renal tissue (Liu and Meng, 2005; Bowe et al., 2017). This last hypothesis is presented as the most plausible, being supported by the results reported by Miller et al. (2017), who detected inhaled inert gold nanoparticles in the urine 15 min after exposure in humans. Furthermore, our findings are supported by epidemiological data that showed a significant association between exposure to $PM_{2.5}$ and the incident risk of chronic renal failure, as well as a decrease in the glomerular filtration rate, with a propensity towards the development of end-stage chronic kidney disease (Mehta et al., 2016; Bowe et al., 2017).

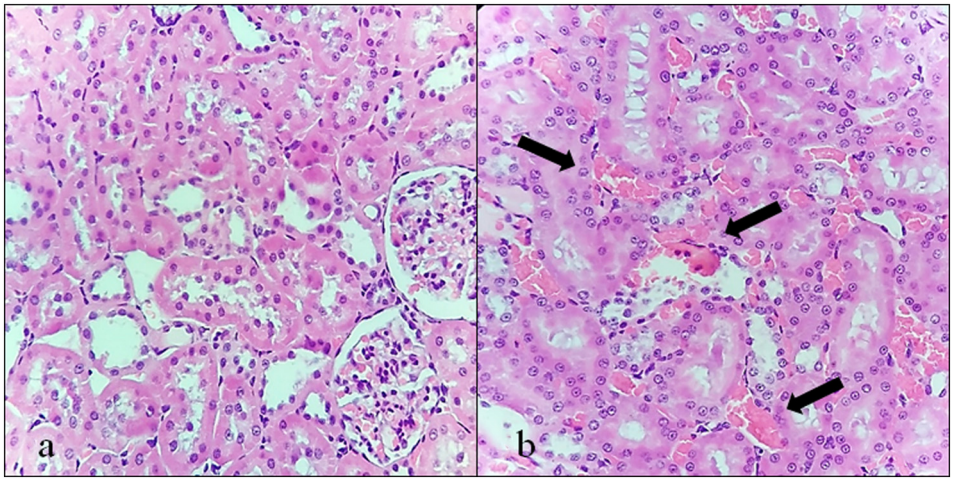

Fig. 5. a, b - Mesangial expansion (PAS, 400×) in SHR. Exposed animals (5b) showed mild to moderate expansion in comparison with controls (5b).

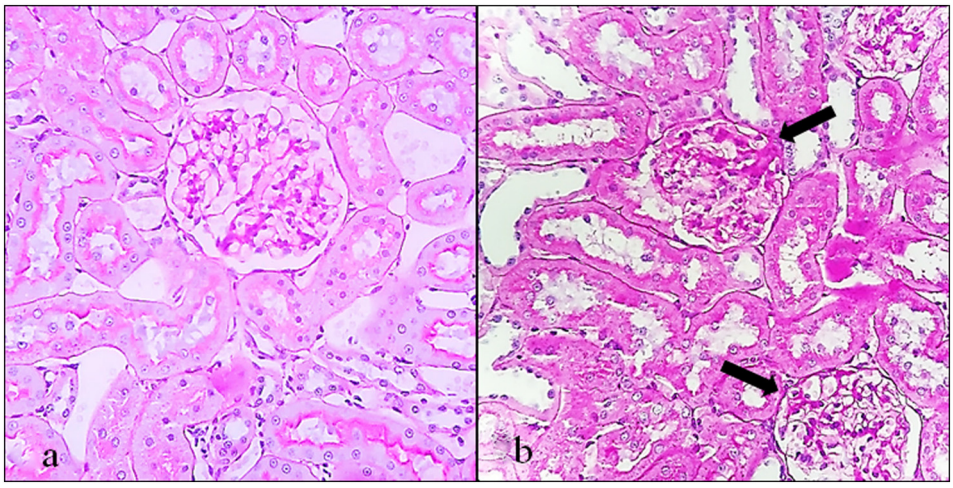

Fig. 6. a, b - Vascular glomerular capillary congestion (HE, 400×) exposed (6b) SHR. The phenomenon was absent in almost all control animals (6a).

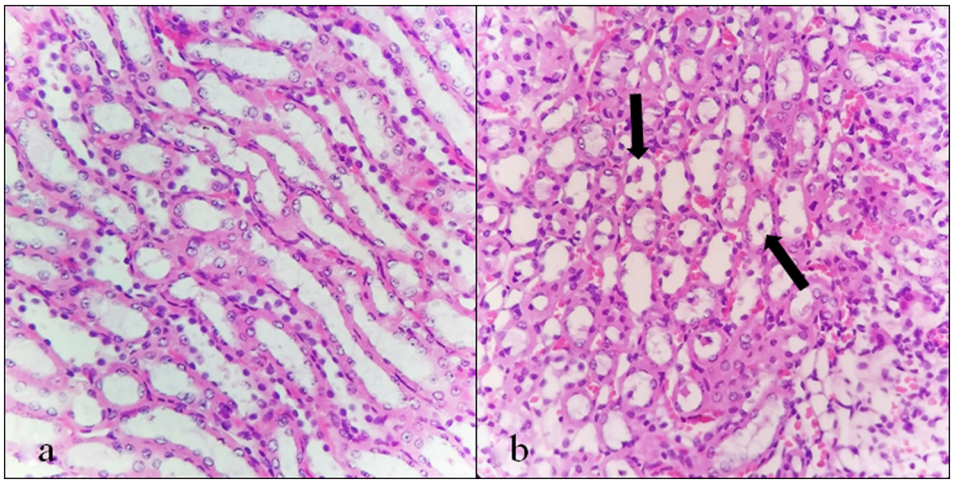

Fig. 7. a, b - Tubular epithelial cells detachment (HE, 400×) in exposed (7b) SHR. Notice there are no cell in tubular space in control animals (7a). Also, a marked eosinophilia can be observed in the tubular cells.

4. Conclusions

In this study we found good evidences reinforcing the notion that sub-chronic exposure to $PM_{2.5}$ may lead to histological alterations in renal tissue that it could be linked to an early stage of renal dysfunction and that could be enhanced by hypertension. The biochemical changes observed in renal tissues also support these results. These outcomes are noteworthy considering that during the exposition, the particles concentrations were below the WHO reference levels most of the days, suggesting there is no safe $PM_{2.5}$ threshold levels in urban

Table 3
Serum determinations en WK and SHR animals (mean ± SE).

Strain determination	W-K			SHR		
	Control	Treatment		Control	Treatment	
Glycemia (mg dL^{-1})	269.0 ± 52.8	253.3 ± 40.8		211.3 ± 25.7	230.0 ± 27.7	
BUN (mg dL^{-1})	21.1 ± 2.1	28.1 ± 1.9	*	23.3 ± 1.7	27.3 ± 1.6	*
Uric acid (mg dL^{-1})	2.06 ± 0.14	2.53 ± 0.64		2.12 ± 0.37	2.22 ± 0.29	
Creatinine (mg dL^{-1})	0.54 ± 0.07	0.59 ± 0.06		0.51 ± 0.02	0.50 ± 0.02	
Blood proteins (mg dL^{-1})	6.07 ± 0.19	5.87 ± 0.39		6.10 ± 0.20	6.40 ± 0.15	

Ref.: "*", $p < 0.05$; "**", $p < 0.01$; "***", $p < 0.001$.

environments.

We showed that sub-chronic exposure to urban $PM_{2.5}$ may induce fibrosis, mesangial expansion, decrease glomerular and tubular lumen volumes and BUN elevation. We also showed that hypertensive animal displayed much more severe alterations than healthy animals, indicating that hypertension is a strong risk factor for the development of diseases related to $PM_{2.5}$ exposure. Furthermore, our results indicate that the evidences of minimal or small-scale abnormalities can be determinant for renal health preservation. We believe this study may contribute to awareness and further investigations to confirm the deleterious effects of exposure to environmental particles on non-target organs.

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