Environ Sci Pollut Res
DOI 10.1007/s11356-015-4324-y

RESEARCH ARTICLE

The impact of aluminum, fluoride, and aluminum–fluoride complexes in drinking water on chronic kidney disease

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Received: 26 September 2014 / Accepted: 5 March 2015

Abstract It is suspected that drinking water containing fluoride and aluminum results in negative health effects especially on brain, liver, and kidney. In this investigation, the effect of F, Al, and AlF_x complex on chronic kidney disease (CKD) was investigated. Mice were treated either with WHO recommended or slightly higher F and Al levels in drinking water. Treatment solutions contained 0.05–10.0 mg/L of F, 0.08–10.0 mg/L of Al, or 0.07–15 mg/L of AlF_x, and the treatment period was 42 weeks. Blood urea level and creatinine levels were investigated as a measure of malfunction of kidneys. Histopathological evaluations of kidney tissues were carried out to assess the extent of damage that F, Al, and AlF_x complex could cause. It was demonstrated that the treated drinking water containing F and Al with par with WHO or moderately above the WHO levels or AlF_x in low level (0.07–15 mg/L) does not lead to CKD in mice.

Keywords Aluminum · Fluoride toxicity · Chronic kidney disease · Water quality · Environment and public health

Responsible editor: Philippe Garrigues

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Introduction

Chronic kidney disease (CKD) is considered to be a worldwide public health problem and receiving increased global attention because of a significant increase in the prevalence of the disease. Aging, diabetes, hypertension, and nephrotoxic drug usage are found to be the primary cause of CKD risk factors while CKD of unknown etiology (CKDu) is prevalence in some regions of the world especially in African, Central American, and Asian nations (Nahas et al., 2005; Codreanu et al., 2006). The term "unknown etiology" is referred, because the disease is not associated with any known risk factors but suspected to be occupational and environmental causes (Soderland et al. 2010). Hence, the major factors that were considered for CKD causes are as follows: environmental toxins (Wanigasuriyaa et al. 2011), heavy metals (e.g., Cd, Pb), (Bandara et al. 2010; Wanigasuriya et al., 2011), fluoride, aluminum, and aluminofluoride (AlF_x) complexes in water (Ileperuma et al. 2009). Though all these risk factors can be categorized into a waterborne factor, presence of Al and F was the most common problem worldwide due to presence of dissolved fluoride and aluminum in drinking water. Despite the toxicity effects of F (Schiffl 2008) and Al (Sargazi et al. 2001) on CKD patients having shown, it has not been clearly demonstrated that F and Al as causative factor (s) in prevalence of CKD. In this investigation, we attempt to elucidate the role of F, Al, and AlF_x complexes in triggering of CKD.

The known toxicity of F, Al, and AlF_x complexes on humans and animals is briefly reviewed in the following paragraphs. Fluoride is an essential element for human beings for development and growth. While it is beneficial at low recommended doses, its toxicity at higher levels has also been well established. As F gets accumulated in hard tissues of the body, endemic skeletal and teeth fluorosis are known at higher F

Published online: 20 March 2015

levels (Chandrajith et al. 2010). In addition, it has shown that there were thyroid changes, growth retardation, kidney changes, and even urolithiasis at high F concentrations (Dhar and Bhatnagar 2009). Kidney damage has been reported after treating 500 ppm of sodium fluoride in the diet of rats for 56 days, and the damage is prominent in young rats than in old rats (Pindborg 1957). High intakes of F lead to degeneration of vascular, glomerular, and tubular degeneration leading finally to interstitial fibrosis affecting the filtering units of the kidneys. Similarly kidney damage with severe renal histological changes as well as increased renal cell apoptosis has been reported for pigs treated with 100 and 250 ppm F (Chattopadhyay et al. 2011). In a similar experiment, mice treated with relatively high (15 and 150 ppm) doses of F showed severe alternations in kidney function as well as kidney architecture (Kawahara 1972). As described above, occurrence of permanent damage to the kidney with higher dose treatment of fluoride is convinced. However, in contrast to higher dose F treatments, the effect of lower dose fluoride treatments with respect to kidney failures is controversial and not apparent, due to lack of in-detail investigations. That is, when monkeys treated with drinking water containing 5 ppm fluoride showed cytochemical characteristics which was interpreted in terms of deleterious metabolic effects in the kidneys (Sohan et al. 1975). On the contrary, a study by Schlesinger et al. revealed no metabolic effects in kidneys treated with 1.5 ppm fluorinated drinking water (Schlesinger et al. 1956). In a recent similar investigation by Xiong et al., it was further demonstrated that there is no difference in protein or albumin levels in children exposed to 0.61 to 5.69 ppm F. However, in the same study, analysis of tubular epithelial cell revealed that over 2 ppm of F was suspected to harm the kidney function in children (Xiong et al. 2007). In a recent investigation by Kobayashi et al., in which rats treated with drinking water containing 0 (control), 5, or 50 ppm F for 60 days were showed, there is no damage induced by F in the 5 ppm treated rats while a vascular congestion was observed on the 50 ppm F treated rates (Claudia et al., 2009). In another experiment, rabbits were injected with 5, 10, 20, and 50 mg NaF/kg body weight/day and shown no kidney damage at low F doses, but severe kidney damage was observed with high-dose treated rabbits ⁽Shashi et al. 2002). Xiong et al. surveyed the dose–effect relationship between drinking water with F levels and damage to kidney functions and reported that drinking water with F levels over 2.0 mg/L can cause damage to kidney functions (Xian et al. 2007). Details of F and health effects have been explicitly described in a review article by Choubisa (Choubisa, 2012).

Unlike F, Al is not an essential element for human beings, but Al is extensively used in modern daily life, and hence, it can be toxic in excessive amounts. Al is a well-known neurotoxic agent, and there is a strong connection between Al and Alzheimer's disease as well as link to other neurological conditions such as Parkinson's and multiple sclerosis Deepa and Shilpi (2011). Though acute Al toxicity is extremely rare, toxicity of Al is usually found in patients with impaired renal function. Additionally, Al poisonings may lead to Al-induced bone disease and lung and brain cancer (Berthon 1996; Corain et al., 1996; and Parkinson et al. 1979). The human immune system is also found to be sensitive to Al exposure (Zhu et al. 2013). It has been shown that Al accumulates in various tissues such as the brain, liver, and kidney, and associated toxicity has been reported. However, in-depth investigations of Al toxicity on kidney function have not been reported comparatively. Even though Al toxicity on the brain, liver, and kidney is reported, most of the investigations were based on higher dose Al toxicity. Al gets easily into the human body, due to the fact that many municipal water supplies are treated with aluminum sulfate and aluminum fluoride, and ubiquitous use of Al in our daily life. Al in our body is excreted by the kidneys, and hence, excessive Al amounts can impair kidney function. According to WHO, the tolerable weekly intake of Al is ~7 mg/kg of body weight for adults (WHO Tech Rep Ser., 1989). By treating 6–10 mg of Al to healthy and kidney-impaired rats, no renal abnormality has been reported with healthy rats while Al toxicity was noted for kidney-impaired rats (Thurton et al. 1972). Similar elevation of renal failure due to Al toxicity has been reported for rats with kidney malfunction or hepatic damage (Cannata and Fernandez 2002 and Mathieu et al. 2005). In vitro studies of Al-induced toxicity on kidney by Sargazi et al. confirmed that there was a slight toxic effect on kidney proximal tubular cells for rats treated with 100 mM/L Al (Sargazi et al. 2001) while a notable kidney damage was reported for rats treated with high doses of Al (Dhar and Bhatnagar 2009 and Jain et al., 2009). Chagnac et al. reported that among the mice treated with high dose (2 mg/day) and low dose (0.2 mg /day) of Al, no pathological changes in kidney were exhibited with low-dose-treated rats (Chagnac et al. 1987). It was noted that the most of reported investigations of Al toxicity on kidney function were either on higher dose treated or kidney-impaired rats. However, overall, a chronic exposure of Al was found to be toxic for patience with CKD (Wills, Savory., 1989). The effect of long-term exposure of lower dose (WHO recommended level) and higher dose (10 mg) of Al on kidney function as a triggering factor for the CKDu is unknown and yet to be determined.

Until recently, about the toxicity of Al-F complex was not known. The discovery of a new class of phosphate analog AlF_x (aluminofluoride complexes) has led to numerous laboratory studies of the hidden danger of AlF_x, and their long-term action for human health is not yet fully identified. Many municipal water supplies are treated with both aluminium sulfate and aluminum fluoride, and even natural water contains Al and F. These two chemicals can also combine easily in the blood to form aluminum–fluoride complex, and body excretion of Al and F depends on their concentrations (Chiba et al.,

2002). Thus, various laboratory studies demonstrated that AlF_x interacts with the all known G protein (guanine nucleotide-binding proteins) activated effect or enzymes. It is evident that AlF_x is a molecule providing false information, which is amplified by processes of signal transmission (Blackmore et al., 1985, Strunecka et al. 2002). The physiological and biological actions of AlF_x in the liver (Strunecka 2002), kidney (Kessabi et al., 1986), brain (Kaur et al., 2009), and blood cells (Rendu et al. 1990) have been demonstrated, and the long-term action of AlF_x may represent a serious and powerful risk factor in human. In a study carried by Varner et al., they observed deposition of Al in the kidneys of rats and postulated role of AlF_x complex in the transport and penetration of Al across the blood–brain barrier (Varner et al. 1998). A series of investigation have proven that AlF_x is a brain toxin and neurotoxic (Varner et al. 1998). In a recent study by Ileperuma et al., (Ileperuma et al., 2009) they demonstrated leaching of 29 and 1.20 mg/L of Al at pH 3.02 and neutral pH, respectively, in the presence of 6 mg/LF when water is boiled in low-quality aluminum utensils. The same authors argued that the formation of aluminofluoride complexes in drinking water may play a significant role in CKDu. However, the toxicity effect of AlF_x complex on kidney and CKD has been not investigated in detail. Zhou et al. investigated the effect of AlF_x complexes on the kidney and suggested that AlF_x can affect the activity of many other ion channels and enzymes in the kidney (Zhou et al. 1990). In another study, it was demonstrated that Al may act synergistically with F to trigger the Alzheimer's disease. The study showed that some of pathologic changes associated with Alzheimer's disease are not induced by Al alone, but by the AlF_x complexes (Lubkowska et al., 2002).

Though there are strong evidence to demonstrate the neurotoxicity of AlF_x, there is no firm evidence to assert that the drinking water containing fluoride (1–10 mg/L) and Al (1–10 mg/L) or AlF_x (0.07–15 mg/L) increases the risk of developing CKD due to lack of in-depth investigations, despite the selected evidence showing that high F in drinking water leads to certain abnormalities in the kidney (Kaur et al., 2009; Zhan et al. 2006). In this investigation, we carried out an animal trail to ascertain the relationship among F, Al, and AlF_x complex on CKD. This investigation might also answer whether the presence of F, Al, and AlF_x complexes in drinking water could be the reason for the CKD of unknown etiology prevailing in some part of Sri Lanka.

Materials and methods

Sample Sixty imprinting control region (ICR; white) female mice 7–8 months of age at the start of the treatment schedule were used. The animals were divided into ten groups (six mice/group). Ten cages were used to house them with adequate space. All subjects were stock colony at Veterinary Research Institute (VRI) and maintained on a 12:12 h light:dark schedule. Food was provided ad libitum (commercial broiler starter). Water was also available ad libitum. Treatment consisting of double-distilled drinking water (ddw) was used as control. For the rest of the groups, water samples with either Al and F separately or Al/F mixtures (in ddw) buffered to pH of 7.0 were used. Exposure to the treated water continued for 42 weeks. During the study period, animals were examined routinely for visible clinical signs (reduced appetite, immersion, etc.) and behavioral changes (water intake, feed intake, cannibalism, etc.).

Preparation of treatment solutions For the ten treatment groups of mice, the following strengths of solutions in ddw were given for the period of 42 weeks. For the preparation of Al concentrations, 1.00 g/L Al standard (1000 mg/L Al in 2 % nitric acid—Fluka, Sigma-Aldrich Chemicals) with serial dilution was used. For the F concentrations, 1000 mg/L NaF (standard solution of Dionex, Thermo Scientific) with serial dilution was used. Groups were divided in to ten groups as shown in Table 1. AlF_x forms spontaneously in aqueous solutions containing fluoride and traces of aluminum ions. Here the Al, F and, the mixtures of Al and F concentration series were decided based on the previous water analysis study in CKD prevalence areas in Sri Lanka, as well as the drinking water level for F and Al with par with WHO level and moderately above the WHO level. In addition, the consumption rates of testing solutions by mice were monitored during test period.

Sample collection and gross examinations At the termination of the experiment, the body weight of mice was measured, and all surviving animals were anesthetized with 5 % ketamine hydrochloride by injecting intramuscular dose (150 mg/kg body weight) ("Ketamil," Troy Laboratories Pty

Table 1 Experimental details of test groups

Group no.	Al (mg/L)	F (mg/L)	Duration (days)	Days of autopsy
1	0.08	0.05	294	295
2	0.08	1.5	294	295
3	0.08	10	294	295
4	0	0.05	294	295
5	0	1.5	294	295
6	0	10	294	295
7	10	0	294	295
8	10	0.05	294	295
9	10	10	294	295
10	ddw (control)	294	295	

Ltd, NSW 2164, Australia). Ten minutes later, heart blood of each animal were collected to micro centrifuge tubes (Kamstrupvej 90, Denmark) and stored at −40 ° C. Carcasses were examined for external changes such as emaciation and dull coat, etc. Then the kidneys were examined for gross changes such as size variations, shape, appearance, and the presence of necrotic or degenerated areas, etc. These organs were separated from the carcasses and fixed in 10 % formal saline for histological processing.

Histopathological evaluations

The tissue samples from the kidney, liver, heart, and lungs fixed in 10 % buffered formal saline were dehydrated and embedded in paraffin wax using an automatic tissue processor and sectioned at 4 μm using a tissue microtome. The microsections were stained with hematoxylin and eosin (H & E) and subjected to histopathological examination under a light microscope. All the histopathological studies were conducted by three individuals, and two of whom were always blind to the treatment of mice from which the tissues were collected. The lesions of CKDu reported by previous workers, viz., inflammations, degeneration, necrosis and fibrosis of the relevant tissues were considered in the histopathological evaluation.

Determination of Al and F content in the kidneys

Tissue samples were digested with Ultrex HNO_3 (microwave wet digestion) and analyzed with graphite furnace atomic absorption spectrometer (AAS, GBC GF 3000, Auto sampler GBC PAL 3300). This approach detects both free and bound Al. For the Al determination, by using GF-AAS, λ=396.2 nm, lamp current=10 mA, slit width 0.5 nm, and deuterium background subtraction were used. The standard SPADNS method was used to determine fluoride ion concentration. The SPAD NS method for fluoride determination involves the reaction of fluoride with a red zirconium dye solution. The spectrophotometer was adjusted for wavelength at 580 nm throughout the procedure.

Results and discussion

At the termination of the experiment, the average body weight of mice was found as 30 g, and each mouse has consumed ~7.0 mL/day of treatment solution. According to WHO report, a recommended F concentration in drinking water is 1.5 mg/L assuming that an adult person consumes 2 L of water per day (Mamczar et al., 2005). Hence, an adult with an average body weight of 60 kg consumes 0.05 mg/kg/day of F. As such, it can be considered that 0.05 mg/kg/day of F is a safe level for F for human being. Similarly, considering the secondary WHO standard for Al (0.05–0.20 mg/L) in drinking water, 0.007 mg/kg/day of Al can be considered as the safe level. Based on the body weight of mice, consumed treated solution and the concentration, the consumed Al and F amounts by mice in each group are listed in Table 2. According to the results presented in Table 2, groups 1 and 9 can be categorized as typical and extreme conditions for both Al and F levels, respectively. In groups 2 and 3, Al amounts were typical while F amounts were moderate and extreme, respectively. In groups 4, 5, and 6, Al level was comparable to control while F levels are typical, moderate, and extreme correspondingly. In groups 7 and 8, Al levels were extreme while F levels were comparable to control and typical, respectively.

When mice were treated with the above test solutions, behavioral and other visible signs such as reduced or loss of appetite, depression, lethargy, emaciation, and dull coat were not observed in any of the mouse in each group. General appearance and behavioral patterns (including appetite and water intake) of the animals in all treatment groups did not differ with one another throughout the testing period.

The number of live–death counts of animals during the test period is given in Table 2. Ten deaths were observed out of 60 mice in groups 1, 2, 3, 5, 8, and 9 within the first 2 weeks of the experiment, while three deaths were observed in groups 2, 3, and 7 in between 35 and 38 weeks of the study period. No other deaths were observed throughout the period of the experiment. Post-mortem findings indicated that the deaths reported in the study period in groups 2, 3, and 7 were due to the old age as the lifespan of ICR mice was ~2 years (http://www.nlac.mahidol.ac.th/nlacmuEN/p_animal_mouse.html). The eight deaths observed in the first few weeks are found to be

Table 2 Live–death counts of animals in ten treatment groups at the end of 42 weeks of the feeding period

Group no.	Al mg/kg/day	F mg/kg/day	Levels of Al and F	No of mice at the end	
				Live	Dead
1	0.019	0.012	Al-T, F-T	5	1
2	0.019	0.35	Al-T, F-M	3	3
3	0.019	2.333	Al-T, F-E	4	2
4	0	0.012	Al-C, F-T	4	2
5	0	0.35	Al-C, F-M	5	1
6	0	2.333	Al-C, F-E	6	0
7	2.333	0	Al-E, F-C	5	1
8	2.333	0.012	Al-E, F-T	4	2
9	2.333	2.333	Al-E, F-E	5	1
10	0	0	Al-C, F-C	6	0

T typical, *M* moderate, *E* extreme, *C* control)

due to stress, as animals have stress as a result of changing of the cage, place, heard, feed, feeding pattern, etc. The other two deaths in group 4 were found to be due to pneumonia. Importantly, post-mortem investigation revealed that these early deaths were not associated with CKD, and evidently, these deaths have no effects from the treatment solutions. This can be further strengthened by applying "Friedman test" statistics to the live–death count of mice (in all the treatment groups). Friedman test gave $p=0.884$, $p=0.850$ (adjusted for ties)>0.05 (p value); all treatment effects were zero. These results clearly indicated that the treatment groups have no correlation with CKD. In other words, all treatment groups have the same affect (s), to the live and death counts of the mice. After euthanasia, carcasses were examined for external lesions. Internal organs especially the kidney, liver, heart, and lungs were examined for gross and microscopic changes, and none of the animals found to have external lesions in relation to the chronic kidney disease. In all treatment groups as well as in control group, kidneys were free of gross lesions.

Blood urea nitrogen (BUN) and creatinine levels in blood are usually employed as an identification method of CKD where BUN is a form of nitrogen present in the blood and the level of urea is a rough estimation of kidney function, where increase in BUN value usually indicates decreased renal function. However, it is known that bleeding in the intestines and congestive heart failure also found to be affected the BUN level. Similarly, creatinine is one of the protein constituents of blood, and it is derived from the nonenzymatic interconversion of creatine in the skeletal muscle. It is a waste product that is made when the body breaks down or when muscles are injured (http://hilltoplabs.com/public/swissblood.html). High blood serum creatinine level means probably kidney damage, but creatinine levels may vary somewhat, even when the kidneys work normally. Creatinine and BUN values of the mice in ten groups were analyzed after 42 weeks and given in Table 3. The average BUN level of the control group (group 10) is found to be ~42 (mg/L), and in all other test groups, the BUN level is in the ~42–45 (mg/L) range. These results indicate that the mice subjected either to typical (0.016 F or 0.019 Al mg/kg/day), moderate (0.35 F or 0.019 Al mg/kg/day), or extreme (2.33 mg/kg/day) F and Al concentrations have no adverse effect on their kidney functions. However, BUN values reported in this investigation in all groups including the control group were in the range 40–51 mg/L which was higher than the BUN level of the reference value of ICR mice (21–26 mg/dL) (http://www.nlac.mahidol.ac.th/nlacmuEN/p_animal_mouse.html). The high BUN values reported in this investigation could be due to high-protein diet given to mice as high-protein diet also results in high BUN level (http://hilltoplabs.com/public/swissblood.html). Similar to BUN values, the creatinine levels of all test groups and the control group were in 0.7–1.4 mg/L range. The reported average standard creatinine levels in mice are found to be in the range of 0.2 to 0.9 mg/dL. (http://www.ahc.umn.edu/rar/refvalues.html). However, according to report 3, the average standard creatinine level may vary due to age, sex, breed or strain, sampling technique, and testing methodology; as such, the range limits should be used as only guidelines (http://www.ahc.umn.edu/rar/refvalues.html). As given in Table 3, the creatinine level of control group is 0.833 while in test groups, it varies in the 0.7–1.4 mg/dL range. Interestingly, it was found that the average creatinine and BUN values of mice in group 9 (highest Al (10 mg/L) and F (10 mg/L concentrated treated group), were 0.8 mg/dL and 41.8 mg/dL respectively. Also, the average creatinine and BUN values of mice in group 1 (lowest Al (0.08 mg/L) and F (0.05 mg/L concentrated treated group), were found to be 0.7 mg/dL and 45.3 mg/dL respectively while in the control group, the average creatinine value was 0.8 mg/dL, whereas the average BUN value was 41.8 mg/dL (Table 3). Hence, in this investigation, the creatinine levels of mice treated with both high- and low-concentrated Al and F were comparable with the values of control group. Furthermore, the creatinine levels of these two extreme groups and the control group were within the baseline levels of creatinine (Fig. 1).

However, we noted that the average creatinine levels of some samples were raised slightly from the baseline creatinine level which may be due to hemolytic effect of blood in some samples during blood withdrawal. Furthermore, absence of any significant difference in creatinine levels in blood serum and BUN values between test and the control groups further substantiates that the kidney functions are not deteriorated or malfunctioned after feeding of mice with typical/lowest (0.016 F or 0.019 Al mg/kg/day), moderate (0.35 F or 0.019 Al mg/kg/day), or extreme (2.33 mg/kg/day) F and Al concentrations. Bonferroni statistical test was performed for BUN and creatinine values to estimate the significant difference among the groups.

Table 3 Average BUN and Creatinine values of the mice in ten groups, at the end of the experiment

Group	Average BUN (mg/dL)±STDEV	Average creatinine (mg/dL)±STDEV
1	45.3±1.3	0.7±0.1
2	41.5±1.4	1.3±0.4
3	44.9±1.0	1.0±0.2
4	45.1±0.9	1.4±0.2
5	42.7±1.3	1.3±0.2
6	41.9±1.3	1.3±0.3
7	43.3±1.1	1.3±0.2
8	41.0±1.1	0.8±0.1
9	41.3±1.0	0.8±0.1
10	41.8±1.1	0.8±0.1

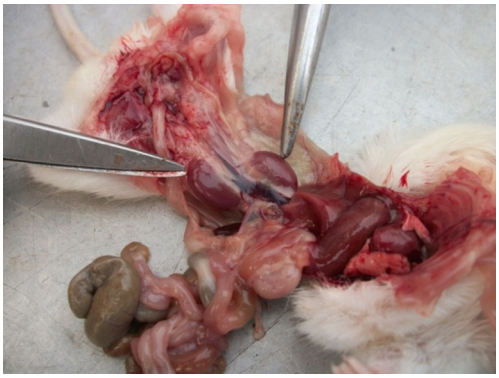

Fig. 1 Dissected carcass showing the absence of gross changers in the internal organs of a mouse in a treatment group (group 9)

Histopathological studies of the kidney tissues of treated mice and the control group were carried out to assess the changes/damages to the kidney tissues caused by F, Al, and AlF_x complex. Images of kidney tissue sections from test groups and the control groups are shown in Fig. 2. In all the test groups and the control group, kidney tissues were devoid of relevant lesions. Since there are no lesions in test groups, compared to control group, any grading or rating system was not implicated in this investigation. Hence, absence of histopathological changes in kidney tissues such as degeneration, necrosis of glomeruli and tubules, atrophy of glomeruli and glomerular capsules, and tubular dilation with leakage that are related to CKD (Jennette et al., 2007) indicative that the kidneys were not affected after feeding the mice with either typical (0.016 F or 0.019 Al mg/kg/day), moderate (0.35 F or 0.019 Al mg/kg/day), or extreme (2.33 mg/kg/day) F and Al concentrations. The histopathological results presented in Fig. 2 clearly demonstrated that the mice fed with drinking water containing 0.05–10.0 mg/L F, 0.08–10.0 mg/L Al, or 0.07–15 mg/L AlF_x were not leading to CKD.

These histopathological results together with the observed BUN and creatinine data of mice treated with F and Al confirm that the drinking water containing 0.05–10.00 mg/L F, 0.08–10.00 mg/L Al, or AlF_x (0.07–15 mg/L) is not a causative factor for CKD in mice. For further substantiation of the above fact, we investigated possible deposition/adsorption of Al and F in kidney tissues of test animals. Thus, we noted the comparable amount of F and Al that were deposited or adsorbed in kidney tissues in control and test groups, i.e., kidneys of mice in groups 6 (0 mg/L Al, 10 mg/L F), 7 (10 mg/L Al, 0 mg/L F), 9 (10 mg/L Al, 10 mg/L F), and 10 (control group, dist. H_2O) which had 14.57, 14.30, 15.12, and 14.85 mg of F/kg (by kidney weight), respectively; also, in the same groups, Al content in kidneys were found be 9.30, 8.33, 9.50, and 8.79 mg/kg, respectively. Hence, it can be concluded that F and Al are additionally not deposited/adsorbed in kidney tissues of mice in test groups compared to the mice in control group. However, deposition of F and Al in kidney tissues has been reported when the mice with CKD were treated with much higher concentrations of Al and F (Brown et al., 2008, Tsunoda et al. 2005). The absence of F and Al deposition in kidneys confirms that the kidneys of the subject mice in this investigation are not damaged or distorted. However, in the study by Vaner et al., the deterioration of kidney function and lesions when the mouse were treated with 0.5–5 ppm AlF_x complex has been demonstrated, but no lesions were observed when mouse were treated with 50 ppm AlF_x complex. Though no reason is given for the observation, it might be due anti-caries protective effect of fluorine (http://www.aapd.org/media/Policies_Guidelines/G_fluoridetherapy.pdf). Similar anti-caries protective effect has been reported when humans were treated with high and moderate amounts of aluminum where high amounts of aluminum were found to depress serum and urinary fluoride levels (Spencer et al. 1981). Interestingly, the same method has been used to reduce fluoride toxicity (Said et al. 1997). Since in this investigation, when mice were treated with AlF_x complex (F amount 0.016–2.33 mg/kg/day), Al amount (0.019–2.33 mg/kg/day, or alumina-fluoro complex AlF_x (0.016–3.43 mg/kg/day), no deterioration of kidney function may indicate that the same anti-caries protective effect may play a role.

In summary, BUN and creatinine values of test groups are comparable with the control group, indicating no adverse effect of Al, F, and AlF_x towards CKD in given concentration series [corresponding to consume F amount (0.016–2.33 mg/kg/day) and Al amount (0.019–2.33 mg/kg/day or alumina-fluoro complex AlF_x (0.016–3.43 mg/kg/day)]. None of the animal was having unusual behavioral or external changes while their organs were free from gross and microscopic changes in relation to CKD. In this investigation, we were able to show that the Al and F ions separately or in complex in water were not supported as causative factors for CKD.

Authors would like to make a special note here regarding CKDu prevailing in some part of the world and Sri Lanka. As mentioned previously, several risk factors/hypothesis have been proposed for the prevalence of CKDu in Sri Lanka such as the following: (a) chronic pesticide exposure and thereby increase of heavy metals (e.g., Cd, Pb,) and As in soil and water, (b) presence of high levels of fluoride in soil and water, (c) presence of high levels of fluoride, aluminum, and aluminofluoride (AlF_x) complexes in water, and (d) cyanobacterial toxins in water (especially in reservoirs) (Dissananayake et al., 2011). By this investigation, we were able to demonstrate that the factors (b) and (c) could not be the major causative factor(s) for the prevalence of CKDu in Sri Lanka.

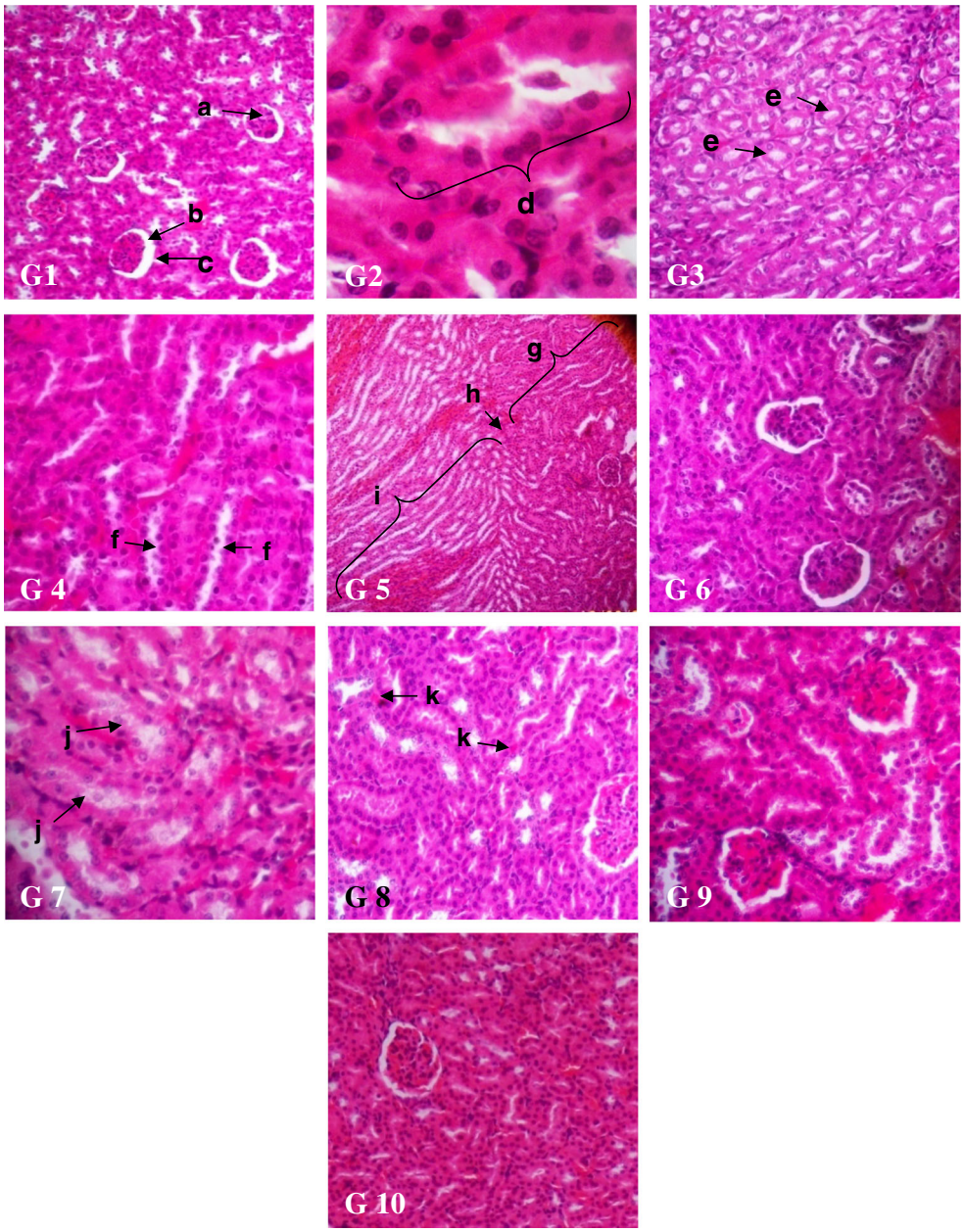

Fig. 2 Histological sections of kidneys showing intact glomerulonephritic tissues collected from mice in all test groups (*G1* to *G9*) and the control group (*G10*). Hematoxylin and eosin (*G1* and *G5*: 40×10; *G2*: 200×10; *G3*, *G4*, *G6*, *G7*, *G8*, *G9*, and *G10*: 100×10). *a* represents the glomerulus, *b* the Bowman's space, *c* the Bowman's capsule, *d* the longitudinal section of proximal convoluted tubules in 200×10, *e* the cross section of distal convoluted tubules, *f* the longitudinal section of proximal convoluted tubules in 100×10, *g* the cortex, *h* the cortico-medullary junction, *i* the medulla, *j* the longitudinal section of distal convoluted tubules, and *k* the capillaries

Authors would like to mention few limitations of this investigation. That is, for the histopathological studies, staining was limited to H & E stain, due to unavailability of special stains for Al, F, and/or AlF_x which might highlight the Al deposition. However, as there are no lesions in subject groups compared to control group, it is unlikely to increase the specificity of findings. Furthermore, withdrawals of blood from available breed of mice were not much smooth, due to miniature blood vessels and very low volume (collected volume 0.5–1.0 mL). Thus, collection of blood for analysis of BUN and creatinine was performed with the heart blood. This process also took prolonged period. Under those circumstances, several collected blood samples could be heamolyzed, in different degrees.

Conclusions

In conclusion, these studies have shown that when drinking water containing fluoride 0.05–10.00 mg/L F and 0.08–10.00 mg/L Al corresponding to consumed F amount (0.016–2.33 mg/kg/day) and Al amount (0.019–2.33 mg/kg/day) or alumina-fluoro complex AlF_x (0.016–3.43 mg/kg/day) were treated with mice, any histopathological structural changes or diminished function of the kidneys were not

observed. Hence, it can be concluded that, in the given concentration series of AlF_x or Al and F ions separately in drinking water do not trigger CKD or not supported as a causative factor for CKD.

Acknowledgments Authors would like to thank D. Aluthpatabendi, Institute of Fundamental Studies, and W. R. Jayaweera, University of Peradeniya, for their contribution.

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