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THE MOLECULAR MECHANISMS OF THE RENAL INJURY IN FLUOROSIS INDUCED BY DRINKING WATER WITH A HIGH FLUORIDE ION CONTENT AND THE EFFECTS OF SELENIUM INTERVENTION

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ABSTRACT: *Objective:* **This study investigated the molecular mechanisms of the renal injury in fluorosis induced by drinking water with a high fluoride ion (F) content and the effects of selenium (Se) intervention.** *Methods:* **A total of 160 newly weaned male Sprague–Dawley rats were randomly divided into three groups: (i) a control group who received tap water; (ii) a F-treated group who received drinking water with 100 mg sodium fluoride (NaF)/L; and (iii) a F+Se-treated group who received drinking water with 100 mg NaF/L and 1.5 mg sodium selenite (Na_2SeO_3)/L. After exposing the rats to the respective drinking waters for 3 or 6 months, we examined for the presence of F toxicity by assessing (i) the renal histology, (ii) the blood biochemical parameters indicative of renal injury, (iii) cell apoptosis, and (iv) the expression levels of the molecules related to the mitochondrial apoptosis signal pathway, namely, cytochrome C (Cytc), caspase-3, and caspase-9.** *Results:* **The F-treated group showed severe renal injury, which was greater at 6 months than at 3 months, and included (i) increased serum creatinine (Cr), serum urea nitrogen (BUN, serum uric acid (UA), malondialdehyde (MDA), and superoxide dismutase (SOD), (ii) decreased reduced monomeric glutathione peroxidase (GSH-Px), and (iii) increased protein expression levels of the apoptosis-related genes Cytc, caspase-3, and caspase-9. Compared to the F-treated group, the F+Se-treated group had significantly less renal injury, apoptosis, and expression of the apoptosis-related proteins.** *Conclusions:* **The changes in the gene/protein expression of the Cytc-caspase-9-caspase-3 apoptosis pathway in the renal mitochondria of the rats with fluorosis may contribute to the molecular mechanisms of F-induced renal injury.**

Keywords: Caspase-3; Caspase-9; Cytc; Fluorosis; Kidney; Selenium.

INTRODUCTION

Chronic fluorosis due to an excess intake of the fluoride ion (F) is a serious disease and its effects may include skeletal fluorosis, dental fluorosis, anaemia, and nervous system damage.[1-3] At present, the theory of free-radical damage is generally accepted in the aetiology of fluorosis-induced adverse effects.[4-6] The kidney is among the target organs of fluorosis.[7] Epidemiological investigations have shown that renal function is significantly affected in patients with chronic fluorosis and that abnormal changes occur in the kidney morphology and ultrastructure of fluorotic experimental animals.[8–10] Rats with chronic fluorosis have been found to have expansion of Bowman's space of the glomerulus, obvious renal tubule dilatation, and NBT/BCIP-staining positive apoptotic cells selectively located in the medullo-cortical junction areas, indicating that excessive F caused abnormal apoptosis of renal tissue cells.[11] By the TUNEL method and flow

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cytometry, Yu et al. found that F poisoning induced significant apoptosis in rat kidney tissue and altered the cell cycle during renal cell proliferation.[12] The results from a previous study by our group demonstrated that F promoted renal tissue apoptosis and the application of selenium (Se) reduced F toxicity by regulating the expression of Bcl-2 and Bax. These results suggest the existence of a linear relationship between oxidative stress and renal cell apoptosis, and imply that abnormal lipid peroxidation is a causative factor for renal cell apoptosis. Anuradha et al. showed that one of the sites of F action in the kidney cells was the mitochondria via increasing oxidative stress and resulting in apoptosis.[13] These findings indicate that F exposure causes renal structural injury and renal dysfunction and that a potential site of action of F is the mitochondria.[14] However, the exact molecular mechanisms involved have not been established.

Se, as a trace element with antioxidant activity, has become of great interest in protecting against oxidative stress. Se is an important component of reduced monomeric glutathione peroxidase (GSH-Px) and some other antioxidant proteins that can eliminate excessive free radicals in the body to restore the oxidation-antioxidation system balance *in vivo*.[15] Animal experiments have shown that Se can be protect the body against the damage caused by F poisoning and thereby alleviate the toxic effect of F.[16] A certain amount of the reversal by Se of F toxicity in rats occurs through promoting the excretion of urinary F, removing free radicals, and improving the antioxidant capacity.[17] However, the exact mechanisms by which Se acts in reducing F toxicity have remained unclear.

The objective of the present study was to provide the basis for the development and application of an anti-fluorine agent by investigating, in rats, the molecular mechanisms underlying F-induced renal injury and the effects of selenium intervention.

MATERIALS AND METHODS

In summary, we:

(i) used the Sprague-Dawley (SD) rat as an animal model,

(ii) observed, by light microscopy, the F-induced morphological damage to renal tissue after 3 and 6 months of F exposure,[18]

(iii) measured the blood biochemical indicators of renal injury to determine the status of both renal oxidative stress and renal cell apoptosis, and

(iv) studied the mitochondrial apoptosis signaling pathway by monitoring cytochrome c (Cytc), caspase-3, and caspase-9.

Experimental animal and treatments: Early weaned, specific-pathogen-free (SPF), healthy male SD rats (160 rats aged 3 weeks) were purchased from the Experimental Animal Center in Zhejiang Province. The animals were randomly divided into three groups after 7 days in laboratory conditions: (i) a control group who received tap water; (ii) a F-treated group who received drinking water with 100 mg sodium fluoride (NaF)/L; and (iii) a F+Se-treated group who received drinking water with 100 mg NaF/L and 1.5 mg sodium selenite (Na_2SeO_3)/L. Tap

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water was used as the solvent for the F and F+Se solutions. Fluoride poisoning was induced by allowing the rats to drink water containing 100 mg NaF/L *ad libitum*. All of the rats were fed with a standard pellet diet (F content< 0.2 mg/kg, Se content of 0.1–0.2 mg/kg) in a well-ventilated feeding room with a room temperature control of approximately 25ºC and a humidity of 50–70%. The bedding material was changed every 3 days. The animals were exposed to the respective drinking waters for 3 or 6 months before being sacrificed and examined.

Determination of the renal organ coefficient and preparation of the renal homogenate: The rats were weighed regularly for the entire duration of the F exposure. At the end of the 3 or 6 month exposure periods, the rats were sacrificed and weighed, and the kidneys were dissected. The isolated kidneys were then rinsed with pre-cooled saline water (4ºC) and weighed. The organ coefficient for the kidney was calculated using the following equation:

$$\text{Kidney coefficient (\%)} \;=\; \frac{\text{Kidney weight}}{\text{Body weight}} \;\times\; 100$$

Parts of the tissue from each kidney were mixed into a homogenate at 4ºC by adding normal saline at a ratio of 1:9 (quality:volume) by a homogenizer.

Measurement of the blood parameters related to renal function: After 3 or 6 months of F exposure, the rats were sacrificed, blood was obtained and allowed to coagulate, and the serum was collected as the supernatant after centrifugation. The serum parameters related to renal function, the serum creatinine (Cr), the serum uric acid (UA), and serum urea nitrogen (BUN) were determined using a biochemical analyzer.

Measurement of the renal tissue biochemical parameters related to renal function: The renal tissues were used for analyzing biochemical parameters related to renal function. Enzyme vitality was assessed by measuring the activities of GSH-Px and superoxide dismutase (SOD) and the level of malondialdehyde (MDA). The total protein in the renal tissue was determined using the bicinchoninic acid (BCA) method.

Morphological observation of renal tissue: The renal tissue was fixed in 4% polyformaldehyde, packed in paraffin and embedded, and then cut into 4 μm-thick paraffin slices. The sections were then conventionally dewaxed and the renal tissue morphology was examined by hematoxylin–eosin staining and light microscopy.

Detection of apoptosis in the kidney: Apoptotic cells were detected by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay using an apoptosis assay kit (BOSTER, Wuhan, PR China) in accordance with the manufacturer's instructions. Tissue slices were dewaxed, treated with 3% H_2O_2 for 10 min to eliminate endogenous peroxidase, and then rinsed with distilled water. Proteinase K treatment for 15 min was then performed and followed by tris-buffered saline (TBS) washing. The slices were subsequently reacted with the labeling buffer, TdT, and DIG-d-UTP mixture for 2 hr at 37ºC and then rinsed with TBS. The slices were then immersed in blocking solution for 30 min. After

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incubation with biotinylated anti-digoxin antibody solution for 30 min at room temperature, the slices were incubated with SABC for 30 min at 37ºC and then stained with DAB after rinsing with TBS. The slices were visualized under a light microscope with positive nuclei presenting with dark-brown staining.

Reverse-transcription polymerase chain reaction (RT-PCR): The total RNA was extracted from kidney tissue (100 mg) with Redzol reagent (SBS Genetech, Beijing, PR China) in accordance with the manufacturer's instructions, and quantified by using Multiskan Spectrum. The RNA purity was assessed by agarose gel electrophoresis. The RNA samples were stored at –80ºC for further analysis. First-strand cDNA was synthesized from 5 μg of total RNA using an RT-PCR Prime Script II first-strand cDNA synthesis kit. β-actin was used as the loading control. The primer pairs adopted are shown in Table 1.

Table 1. Genes and primer pairs

Gene	Forward-primer (5'→3')	Reverse-primer (5'→3')
Caspase-9 (535 bp)	CTTCCTCGCTTCATCTCCTG	AGGTCGTTCTTCACCTCCAC
Caspase-3 (527 bp)	CATGGAAGCAAGTCGATGG	GGGTGCGGTAGAGTAAGCAT
Cytc (540 bp)	GGAGGCAAGCATAAGACTGG	TCAATAGGTTTGAGGCGACAC
β-actin (432 bp)	TCAGGTCATCACTATCGGCAAT	AAAGAAAGGGTGTAAAACGCAG

The reaction conditions were as follows: 95ºC preheating for 5 min; 30 cycles of 95ºC for 30 sec (denaturation); 56º (Cytc), 52.5ºC (caspase-9), 54.2ºC (caspase-3), and 56ºC (β-actin) for 30 sec (annealing); and 72ºC for 40 sec (elongation), followed by 72ºC heating for 5 min. The reaction mixture (20 μL) contained 2 μL of 10×PCR buffer, 2 μL of dNTP mixture, 1μL of Mg^{2+}, 1μL of template, 1μL of Primer 1, 1μL of Primer 2, 0.2μL of Taq E, and 11.8μL of double-distilled H_2O. The PCR products were separated on a 1% agarose gel, stained with Gold View, scanned, and quantified using a gel imaging system and Bio-Rad's Quantity One software. Data were reported as expression ratios calculated from the expression of each target gene divided by the geometric mean expression of the housekeeper gene.

Western blotting: Portion samples from the kidney tissue were obtained. After electrophoresis, film transfer, and sealing, the membranes were incubated at 4ºC overnight with primary antibodies, Cytc (1:300), caspase-3 (1:800), caspase-9 (1:800), and β-actin (1:800). The membranes were rinsed with 1×TBST three times for 10 min each time, followed by incubation with the second antibody of the horseradish peroxidase standard of sheep anti-rabbit antibody at 37º on a shaker. Afterward, the solutions were rinsed with 1×TBST three times for 15 min

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each time. ECL color liquid was used to develop the film which was then fixed. After using the Quantity scanner, semi-quantitative analysis was performed using the software Quantity One.

Statistical analysis: The data are presented as mean±standard error of the mean (mean±SE). One-way analysis of variance (ANOVA) was used to compare the means among the different groups and followed by a *post-hoc* test. Differences were considered significant when $p<0.05$. All the analyses were performed using the SPSS 20.0 software.

RESULTS

Body weights and organ coefficients of the kidneys following 3 and 6 month's exposure: No significant change was found in the body weight and the organ coefficient of the kidney for the rats in the F- and the F+Se-treatment groups, compared with those in the control group, at the end of 3 and 6 month's exposure to the three drinking water conditions, tap water (control), F, and F+Se ($p>0.05$).

Changes in the blood parameters related to renal function following 3 and 6 month's exposure: SERUM CR: After 3 month's exposure, when compared to the control group, there were no significant changes in the serum Cr in the F group and F+Se groups. However, after 6 month's exposure, compared to the control group, the serum Cr level in the F group significantly increased in the F group ($p<0.05$) but not in the F+Se group ($p>0.05$) suggesting that Se protected the kidney from F toxicity (Table 2).

Table 2. Serum creatinine (Cr) levels (µmol/L, mean±SE) in the different groups (n=10)

Group	Serum creatinine (µmol/L, mean±standard error)	
	3 month's exposure	6 month's exposure
Control	37.5±2.878	38.1±3.091
Fluoride (F)	40.0±3.023	46.2±4.367*[†]
Fluoride + selenium (F+Se)	38.5±3.381	39.1±2.167[‡]

*Compared with the corresponding (6 month's exposure) control group: *$p<0.05$;
[†]compared with the 3 month's exposure to fluoride group: [†]$p<0.05$;
[‡]compared with the corresponding (6 month's exposure) fluoride group: [‡]$p<0.05$.

SERUM BUN AND UA: Compared to the serum Cr, the serum BUN and serum UA were more sensitive to F-induced renal toxicity. After both 3 and 6 month's exposure: (i) when compared to the control group, the BUN and UA levels were significantly increased ($p<0.05$) in the F group and (ii) when compared to the F group, the BUN and UA levels were significantly decreased ($p<0.05$) in the F+Se group. No significant differences ($p>0.05$) in the serum BUN level were present in the respective groups after 3 and 6 months of exposure, but the serum UA level was significantly higher ($p<0.05$) after 6 months, compared the value at 3 months, in the F group but not in the control and F+Se groups (Tables 3 and 4).

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Table 3. Serum urea nitrogen (BUN) levels (mmol/L, mean±SE) in the different groups (n=10)

Group	Serum urea nitrogen (mmol/L, mean±standard error)	
	3 month's exposure	6 month's exposure
Control	5.34±0.368	5.55±0.679
Fluoride (F)	6.25±0.641*	6.66±0.691*
Fluoride + selenium (F+Se)	5.64±0.510‡	5.87±0.456‡

*Compared with the corresponding control group: *$p<0.05$;
‡compared with the corresponding fluoride group: $^{‡}p< 0.05$.

Table 4. Serum uric acid (UA) levels (µmol/L, mean±SE) in the different groups (n=10)

Group	Serum uric acid (µmol/L, mean±standard error)	
	3 month's exposure	6 month's exposure
Control	120.971±12.270	125.838 ±11.374
Fluoride (F)	145.538±18.312*	156.650±11.325*†
Fluoride + selenium (F+Se)	127.375±13.997‡	129.088±12.309‡

*Compared with the corresponding control group: *$p<0.05$;
†compared with the 3 month's exposure to fluoride group: $^{†}p < 0.05$;
‡compared with the corresponding fluoride group: $^{‡}p< 0.05$.

Effects of 3 and 6 month's exposure on the cell morphology and structure of the rat kidney: In the control group, after both 3 and 6 month's exposure, the rat kidney tissue structure showed no obvious abnormality in the kidney glomerulus or the renal tubular epithelial cells which were structurally intact and neatly arranged with clear lumina (Figures 1A and 2A).

However, in the F group, after 3 month's exposure, (i) the renal capsule cavities were significantly expanded, (ii) some glomeruli were shrunken with invagination, (iii) the renal tubules were expanded, and (iv) the renal tubule epithelial cells were sparse, disordered, and appeared vacuolar. At end of 6 months, (i) some glomeruli were shrunken, (ii) the interstitial capillaries were expanded with some erythrocyte exudation, (iii) the renal tubular epithelial cells exhibited vacuolar cells and were sparsely arranged, disordered, and even partially exfoliated, (iv) granular and vacuolar renal tubular epithelial cells occurred to varying degrees, and (v) interstitial small blood vessels appeared slightly dilated. Compared to the changes

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present after 3 month's exposure to F, the F-induced renal tissue damage was more severe at the end of 6 months (Figures 1B and 1B).

In comparison to the changes seen in the F group, no significant abnormalities were noted in the structure of the renal tissue of the F+Se group indicating that the addition of Se gave protection from F-induced toxicity (Figures 1C and 2C).
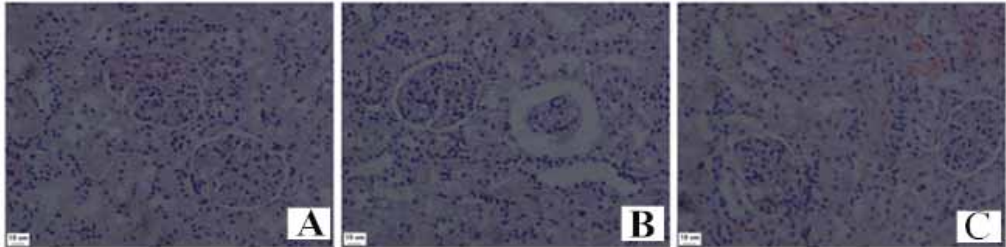

Figure 1. Changes in the morphological and histological structures of the rat renal cortex after three month's exposure. A: control group, B: fluoride group, C; fluoride and selenium group. (HE staining, magnification ×200).
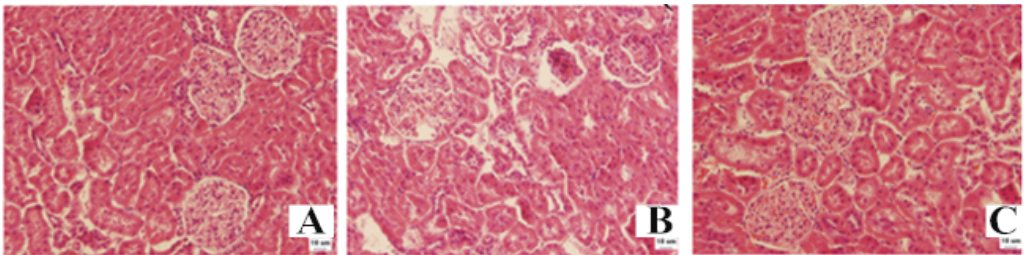

Figure 2. Changes in the morphological and histological structures of the rat renal cortex after six month's exposure. A: control group, B: fluoride group, C; fluoride and selenium group. (HE staining, magnification ×200).

Changes in the renal tissue biochemical parameters following 3 and 6 month's exposure: MDA LEVEL: Compared with the corresponding control group at the end of both 3 and 6 months, the renal level of MDA in the F group was significantly increased ($p<0.01$). However, the MDA levels in the F+Se group were not significantly different from those of the corresponding control group after either 3 or 6 months. The MDA level in the F+Se group was significantly less ($p<0.01$) than that of the corresponding F group after both 3 and 6 months. The MDA level in the F group at significantly increased ($p<0.05$) at 6 months compared to the value in this group at 3 months (Table 5).

GSH-P_X ACTIVITY: Compared with the corresponding control group at the end of both 3 and 6 months, the renal level of GSH-Px activity in the F group was significantly decreased ($p<0.05$) while the values for the F+Se group were significantly increased ($p<0.01$). The GSH-Px activity level in the F+Se group was significantly greater ($p<0.01$) than that of the corresponding F group after both 3 and 6 months. The level of GSH-Px activity after 6 months of exposure was not significantly different ($p>0.05$) to the level after 3 months in the same group for any of the groups (Table 6).

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Table 5. Malondialdehyde (MDA) levels (nmol/mg protein, mean±SE) in the rat kidney
in the different groups (n=10)

Group	Malondialdehyde (nmol/mg protein, mean±standard error)	
	3 month's exposure	6 month's exposure
Control	2.798±0.067	2.923±0.126
Fluoride (F)	4.231±0.327§	4.911±0.175§†
Fluoride + selenium (F+Se)	2.857±0.148$^\parallel$	3.068±0.145$^\parallel$

§Compared with the corresponding control group: §p<0.01;
†compared with the 3 month's exposure to fluoride group: †p < 0.05;
$^\parallel$compared with the corresponding fluoride group: $^\parallel$p< 0.01.

Table 6. GSH-Px (reduced monomeric glutathione peroxidase) activity (value$\times10^{-3}$ U/mg
protein, mean±SE) in the rat kidney of the different groups (n=10)

Group	GSH-Px (value$\times10^{-3}$ U/mg protein, mean±standard error)	
	3 month's exposure	6 month's exposure
Control	2.482±0.105	2.434±0.099
Fluoride (F)	2.058±0.133*	2.018±0.081*
Fluoride + selenium (F+Se)	2.842±0.057$^{\S\parallel}$	2.947±0.131$^{\S\parallel}$

*Compared with the corresponding control group: *p<0.05;
§compared with the corresponding control group: §p<0.01;
$^\parallel$compared with the corresponding fluoride group: $^\parallel$p< 0.01.

SOD ACTIVITY: Compared with the control group at the end of 3 months, no significant differences were present in the activity of SOD in the F or the F+Se groups, although a non-significant decrease was present in the F group. However, compared with the control group at the end of 3 months, the activity of SOD was significantly reduced in the F group (p<0.05) but not in the F+Se group (Table 7).

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Table 7. Superoxide dismutase (SOD) activity (U/mg protein, mean±SE) in the rat kidney of the different groups (n=10)

Group	Superoxide dismutase (U/mg protein, mean±SE)	
	3 month's exposure	6 month's exposure
Control	295.029±11.900	283.302±7.731
Fluoride (F)	279.583±11.432	247.975±3.618*†
Fluoride + selenium (F+Se)	290.603±8.844	275.617±6.039‡**

*Compared with the corresponding control group: *$p<0.05$;
†compared with the 3 month's exposure to fluoride group: †$p < 0.05$;
‡compared with the corresponding (6 month's exposure) fluoride group: ‡$p< 0.05$;
**compared with the 3 month's exposure to fluoride + selenium group: **$p<0.05$.

Detection of apoptosis in the renal tissues: After both 3 and 6 month's exposure, apoptotic cells with densely stained nuclei were rare in the kidney tissues of the control group but significantly increased in the F group. Compared with the corresponding F group, the number of apoptotic cells in the F+Se group, after both 3 and 6 months, was obviously reduced. For the F group, more apoptotic cells were present after 6 month's exposure than after 3 month's exposure indicating that a longer exposure time resulted in more severe renal damage (Figures 3 and 4).

Figure 3. Protection of the kidney by selenium against fluoride-induced apoptosis. The tissue is rat renal cortex and the staining for apoptosis was done using the TUNEL assay after three month's exposure. A: control group, B: fluoride group, C; fluoride and selenium group. (magnification ×200).

Figure 4. Protection of the kidney by selenium against fluoride-induced apoptosis. The tissue is rat renal cortex and the staining for apoptosis was done using the TUNEL assay after six month's exposure. A: control group, B: fluoride group, C; fluoride and selenium group. (magnification ×200).

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Renal expression levels of the mRNA of the apoptosis-related genes Cytc, caspase-9, and caspase-3: Compared to the corresponding control group, after both 3 and 6 month's exposure, the mRNA levels of Cytc, caspase-9, and caspase-3 were significantly increased (p<0.01) in the F group. Compared to the corresponding F group, after both 3 and 6 month's exposure, the mRNA levels of Cytc, caspase-9, and caspase-3 in the F+Se group were significantly decreased (p<0.01, Figures 5 and 6).
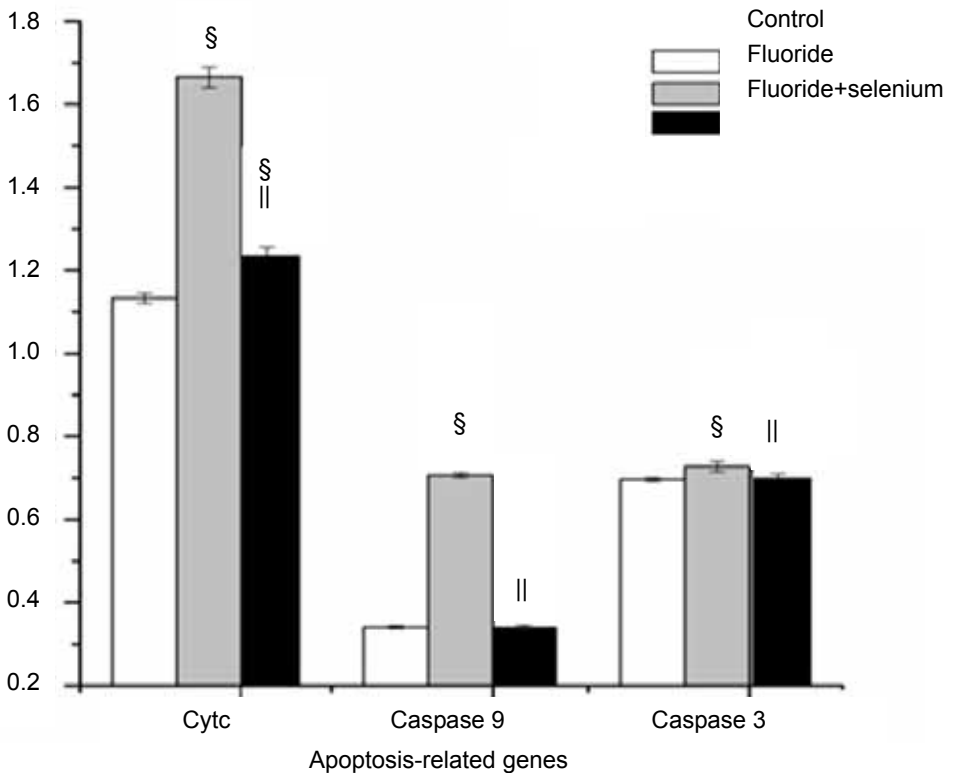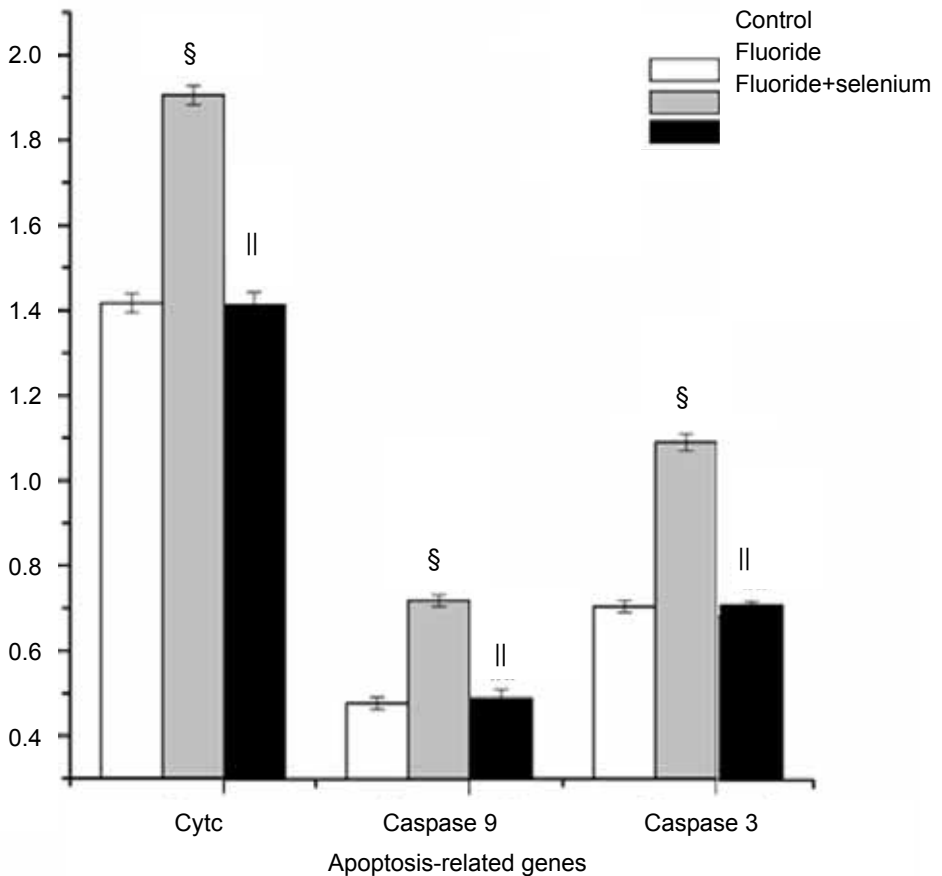

Figure 5. The expression levels of the mRNA of the apoptosis-related genes in the different groups at the end of three months. Data are presented as mean±SE, n=10. §Compared to the corresponding control group: §p<0.01; ‖compared to the corresponding fluoride group: ‖p<0.01.

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Relative mRNA expression of β-actin

Figure 6. The expression levels of the mRNA of the apoptosis-related genes in the different groups at the end of six months. Data are presented as mean±SE, n=10. §Compared to the corresponding control group: §$p<0.01$; ‖compared to the corresponding fluoride group: ‖$p<0.01$.

Renal expression levels of the apoptosis-related proteins of the apoptosis-related genes Cytc, caspase-9, and caspase-3: Compared to the corresponding control group, after both 3 and 6 month's exposure, the levels of the apoptosis-related proteins of Cytc, caspase-9, and caspase-3 were significantly increased ($p<0.01$). Compared to the corresponding F group, after both 3 and 6 month's exposure, the levels of the apoptosis-related proteins of Cytc, caspase-9, and caspase-3 in the F+Se group were significantly decreased ($p<0.01$, Figures 7 and 8).

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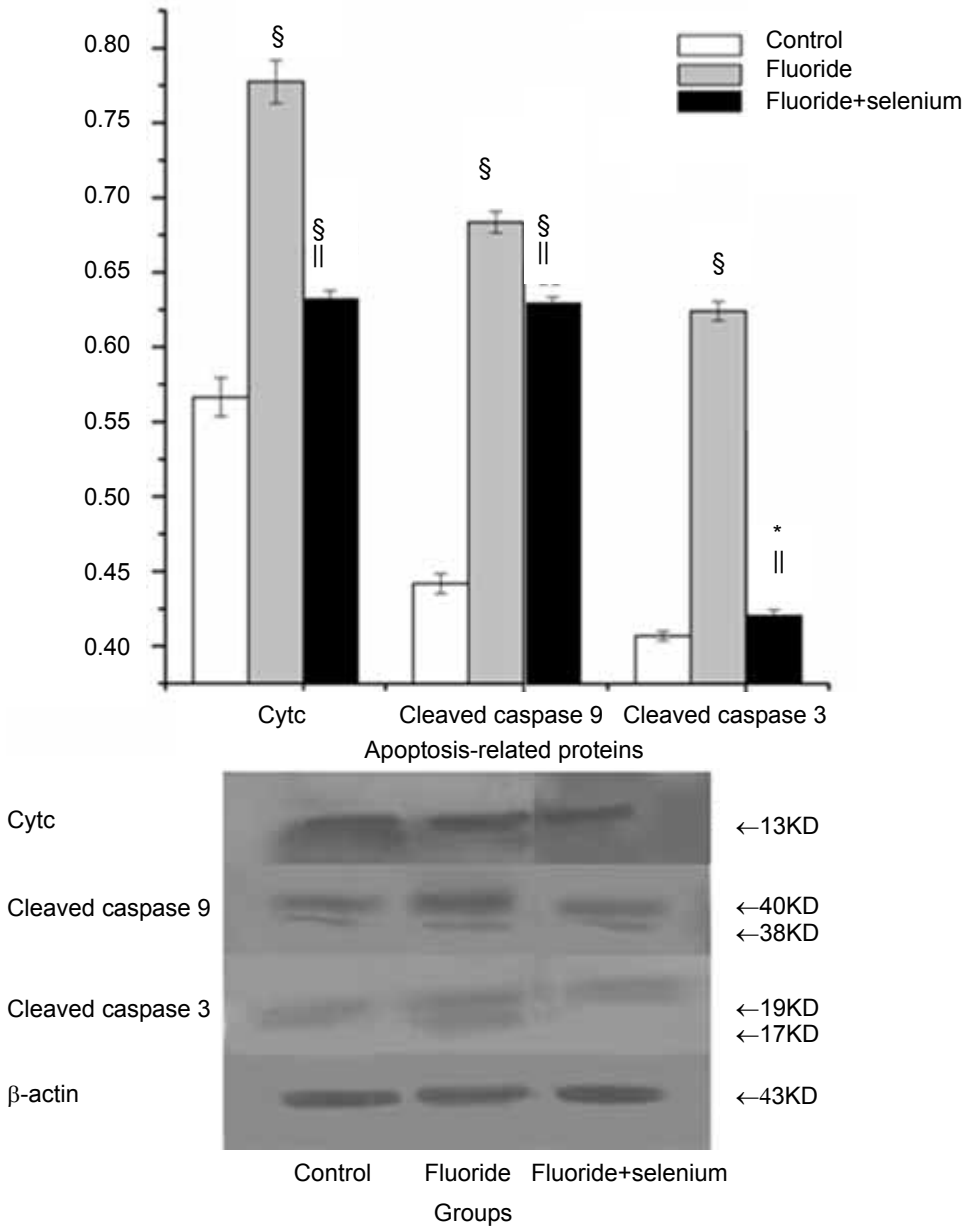Zhang, Liao, Ke, Ouyang, Zhang

Figure 7. The protein expression levels of the apoptosis-related genes in the different groups at the end of three months. Data are presented as mean±SE, n=10. §Compared to the corresponding control group: §p<0.01; ||compared to the corresponding fluoride group: ||p<0.01.
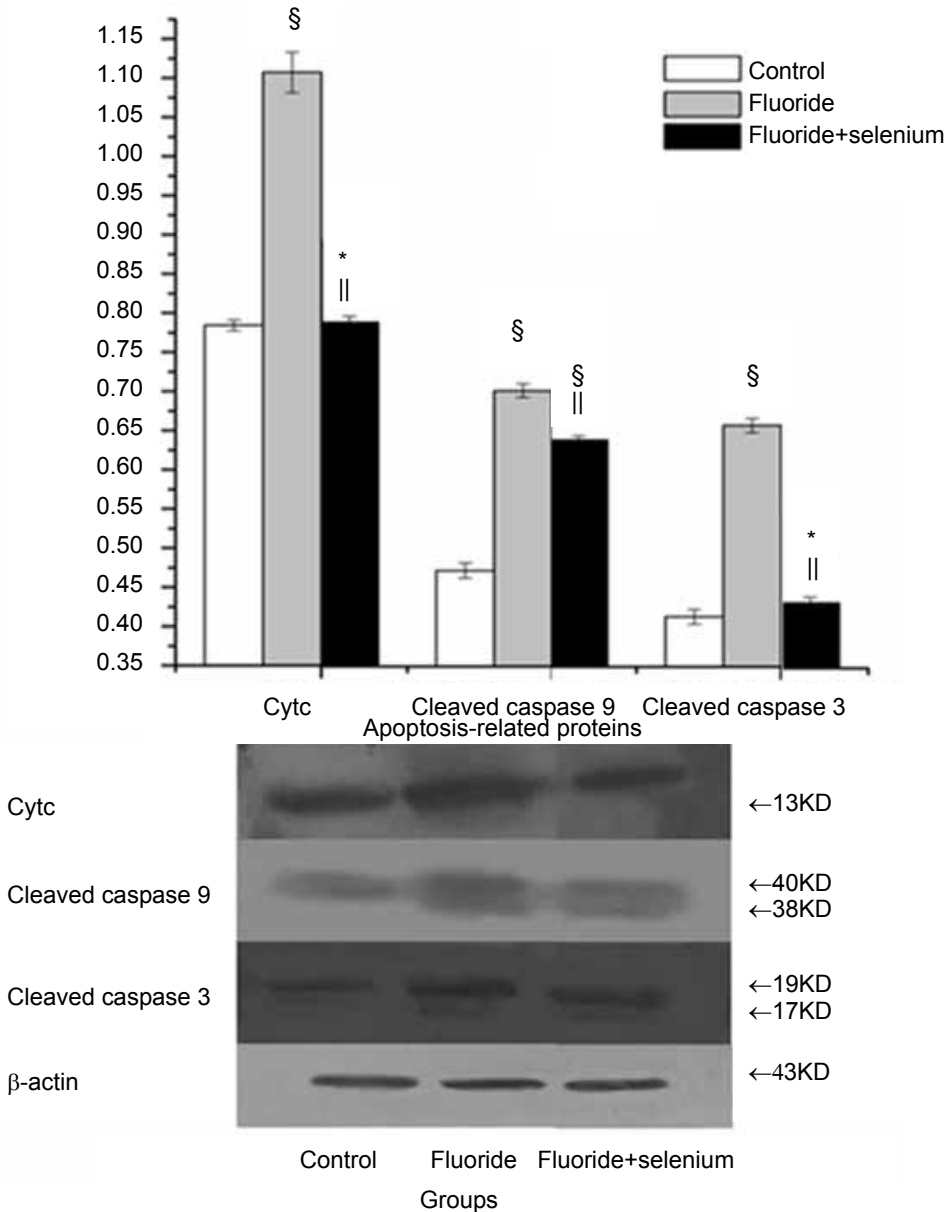
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Figure 8. The protein expression levels of the apoptosis-related genes in the different groups at the end of six months. Data are presented as mean±SE, n=10. *Compared to the corresponding control group: *p<0.05; §compared to the corresponding control group: §p<0.01; ||compared to the corresponding fluoride group: ||p<0.01.

DISCUSSION

The results of the renal image analyses in the present study show that after F exposure in rats the glomerular capsule cavity expanded, granular degeneration and vacuolation occurred, renal tubular ectasia was manifested, and renal tubular

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epithelial cell edema in the corticomedullary junction ensued. The damage to the kidney was reflected in significant increases in the serum levels of Cr, BUN, and UA, and impairments to glomerular filtration and urinary tract patency.

Xu et al. found that SOD, an important antioxidant enzyme in the body, was decreased in patients with endemic fluorosis.[19] MDA is the final product of oxidative metabolism, and changes in its level can reflect the level of free radicals present in an organ. Liu et al. reported that changes in renal lipid peroxidation and renal damage parameters showed a parallel relationship or correlation, suggesting that lipid peroxidation is one of the mechanisms of chronic fluorosis-induced kidney damage.[20] The results of the present study show that fluorosis decreased the antioxidative levels of rats and that the level declined further with prolonged F exposure. These findings provide support for the free-radical damage theory of fluorosis.

Apoptosis is programmed cell death in the body and a response to non-immediately lethal toxic substances. Therefore, abnormal cell apoptosis may be a key link to fluoride-induced damage.[21]

Caspase-induced apoptosis in mammalian cells mainly depends on three pathways: the death receptor pathway, the mitochondrial pathway, and the endoplasmic reticulum pathway. In the process of cell apoptosis, the mitochondria are believed to be the center of the regulation and control of apoptosis, and of the key molecules involved including Cytc. In the present study, we further studied the molecular mechanisms of F-induced renal injury by monitoring the signal molecules of the mitochondrial apoptotic pathway and determining the protein expression levels of Cytc and caspase-3, and caspase-9 in the renal tissue in fluorotic rats by an immunohistochemical method in accordance with the observations of Gao et al. who showed that F exposure can increase the protein expression level of Cytc, caspase-8, caspase-3, and caspase-9.[22,23] Gao et al. suggested that high F levels can promote the apoptosis of renal cells and the death receptor pathway, and that the mitochondrial pathway may be also involved in F-induced apoptosis in rat renal cells.[22,23] The results of the present study are basically consistent with the results of Gao et al,[22,23] who used Western blot and RT-PCR to show that excessive F promoted the abnormal expression in the renal mitochondria of the proteins/genes of Cytc, caspase-3, and caspase-9, and this consequently promoted renal cell apoptosis.

Yuan et al. showed in subjects given a fluorine–selenium combination, by analysis of kidney tissue microstructure and antioxidant levels and the detection of the genes/proteins of the kidney mitochondria apoptosis pathway, that an appropriate dose of selenium can increase the antioxidant enzyme activity in fluorine-poisoned organisms through the indirect role of GSH-Px and SOD.[24] This Se concentration could reduce the toxic effects of F by reversing the fluorosis-induced abnormal expression of Cytc, caspase-3, and caspase-9, and decreasing the degree of fluorine-induced apoptosis. These chronic effects of Se on F poisoning are more obvious than the subchronic manifestations.

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Our results are consistent with the finding by Zheng et al. that selenium, in a concentration of 1.5 mg/L, can be used as a supplement to antagonise the development of F toxicity.[25] Zheng et al. investigated the molecular mechanism of brain impairment induced by drinking fluoridated water and selenium intervention and found that the learning and memory of rats in the NaF group significantly decreased. In their NaF group, there were significant increases in the number of apoptotic cells, the expression levels of Cytc mRNA and protein, and the expression levels of caspase-9 and caspase-3 mRNA while the caspase-9 and caspase-3 protein levels decreased significantly. For the NaF+Se group, compared to the NaF group, there were significant decreases in the mRNA levels of Cytc and caspase-9, and the protein levels of Cytc, while the protein levels of caspase-3 and caspase-9, as well as the mRNA levels of caspase-3, increased significantly. Zheng et al. considered that (i) the mitochondrial Cytc-caspase-9-caspase-3 apoptosis pathway in the hippocampus was one of the mechanisms leading to fluorosis-induced brain damage, (ii) the Cytc signaling molecules were possibly the key target molecules in fluorosis-induced apoptosis, and (iii) selenium could alleviate fluorosis-induced brain injury.

In addition, the results of Zheng et al. varied from those of our previous reports in which we found that drinking Se alone did not damage the hippocampus of the rat brain, and that feeding appropriate amounts of both F and Se caused some antagonistic effects. The work of Zheng et al. suggests that the sensitivity of Se differs among different organs of the body.[25] Therefore, the effect of selenium as an effective anti-fluorine agent needs to be further investigated urgently.

CONCLUSIONS

In conclusion, the molecular mechanisms of the renal damage occurring in the body after the fluoride exposure may include excessive fluoride levels leading to an increase in renal cytolipid peroxidation and mitochondrial damage, the release of Cytc to the cytosol, activation of caspase-9 and caspase-3, the consequent worsening of renal cell apoptosis, and eventually to damage to the renal cells. Selenium supplementation at 1.5 mg/L can prevent the renal cell mitochondrial apoptosis induced by fluoride by blocking the abnormal expression of the genes and the proteins of the signaling pathway molecules Cytc, caspase-9, and caspase-3. Thus Se can be helpful in preventing fluoride-induced toxicity to renal toxicity.

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