Protective role of diet supplements Spirulina and Tamarind fruit pulp on kidney in sodium fluoride exposed Swiss albino mice: Histological and biochemical indices

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Received 19 December 2013; Revised 01 September 2014

Fluoride toxicity through potable water, particularly ground water, is not uncommon in countries such as India, China, Iran, Iraq, Turkey, parts of Africa and Afghanistan. Kidney being the main organ involved in fluoride removal, it accumulates considerable amount of fluoride. Here, we report toxic effects of oral exposure of Swiss albino mice to fluoride (sub-acute: 190 mg/kg body wt. for 7 days; and sub-chronic: 94 mg/kg body wt. for 90 days) and recovery of sub-chronic fluoride exposed mice after 90 days of sodium fluoride (NaF) withdrawal. The role of diet supplements (Spirulina and tamarind fruit pulp @ 230 mg/kg body wt. independently as well as in combination) in amelioration of fluoride toxicity has also been screened. Compared with controls, feed intake decreased from 3-43%, body wt. 4-18%, and kidney wt. 5-12% in treated mice (except diet supplement groups of sub-chronic exposure) while their water intake increased from 4-43%.

Histopathological changes in the cortical region of kidney in fluoride treated mice were as follows: dilation of bowman’s capsule and thickening of its parietal and visceral layer; alterations in glomeruli size and their sclerotization; increase in bowman’s space; proliferation of mesangial cells; reduction in podocyte counts; and dilation of proximal and distal tubules. Fluoride exposure altered tissue biochemistry (protein, acid phosphatase and alkaline phosphatase content) and increased urea (23-58%) and creatinine content (14-127%) in the serum. Sub-acute exposure was found more toxic. The diet modulation not only reduced fluoride toxicity but also led to better recovery of treated mice after withdrawal, especially in combination.

Keywords: Fluoride toxicity, Pollution, Potable water, Renoprotective

Fluoride is a ubiquitous non-metallic trace element in the earth’s crust. Its natural concentration in the groundwater ranges 1-44 ppm. According to WHO1, 1.0 mg/L is the permissible limit of fluoride in drinking water. But groundwater with high fluoride concentration occurs in many areas of the world such as large parts of Afghanistan, Africa, China, India, Iran, Iraq and Turkey. India and China, due to population, are the most affected countries. About 177 districts in India are fluoride affected and Rajasthan alone, has 32 districts identified as fluorosis prone2,3.

Fluoride is considered one of the two major groundwater pollutants3. Fluoride ion passes through the intestinal mucosa passively and enters into blood stream wherefrom it diffuses in to organs through cell membranes4, including brain across blood brain barrier, and placenta5,6. Kidney is the major organ associated with fluoride removal from the body through urinary excretion. Being rich in vasculature, kidney accumulates fluoride, and therefore, exposed to high concentrations of fluoride7.

The toxicity of fluoride to organisms is a cause of great concern because of its increasing availability through various routes, and hence, there is a need to explore suitable strategy to minimize it3,5,6. Neurotoxicity of fluoride as well as the protective role of Aloe vera, Curcuma longa and Ocimum sanctum have been reported earlier8. An earlier study has demonstrated that fluoride induced oxidative stress in kidney can be ameliorated by compounds having antioxidant properties such as vitamin E, alone and in combination with methionine7. Ranjan et al.8 have also demonstrated amelioration of fluoride toxicity by Tamarindus indica and Moringa oleifera extract. Similarly, arjunolic acid from plant sources such as Terminalia arjuna, Combretum nelsonii and Leandra chaetom, has been known to protect hepatocytes from sodium fluoride induced cytotoxicity and necrosis9.

Spirulina growing naturally in the warm climate countries has been found to be a good source of

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protein, minerals, essential fatty and amino acids, vitamins and antioxidant pigments such as carotenoids. Since it is safe for human consumption, it has been considered as a supplement in human food. Besides, it has been found protective to experimental animals exposed to heavy metals, fluoride, azo dye and gentamicin.

Tamarind (*Tamarindus indica* L.) is a tree cultivated widely in different countries including India. Its fresh and dried fruits are used as sour flavoring agent in several Indian cuisines. The fruit pulp contains high levels of antioxidants like polyphenols and flavanoids. Thus, fortifying diet of fluoride treated organism with antioxidant rich supplements and improving kidney efficiency for fluoride removal may be an appropriate strategy to manage fluoride toxicity. Here, we studied renoprotective role of Spirulina and tamarind separately and also in combination on fluoride induced sub-acute and sub-chronic toxicity in Swiss albino mice, including toxicity reversal.

**Materials and Methods**

The investigation was carried out in the following four phases:

**Phase 1**— Sixty healthy young male mice aged 75-80 days, weighing 30 ± 0.5 g were acclimated for one week prior to entry into the experimental protocol and were divided equally into 6 groups (10 mice/group) supplied with different treatments as follows: Group I (sub-acute group) received orally through gavage (0.5 mL/mice) sub-acute dose of fluoride (@190 mg/kg body wt. prepared by dissolving sodium fluoride (Merck Ltd., Mumbai, India) in distilled water) for 7 days whereas of Group II and III (sub-chronic groups) received sub-chronic dose of fluoride (@94 mg/kg body wt.) for 90 days. The respective controls of sub-acute (group IV) and sub-chronic (group V and VI) studies received an equivalent amount of vehicle (distilled water) for the exposure period.

Animals were kept in a well ventilated, noiseless environment (Temp. 24 ± 3°C; humidity 40-60%; 12 h light:dark cycle as per INSA guidelines) and allowed free access to standard chow (Ashirwad Ltd., Chandigarh, India) and potable water (pH 7.1; ER 0.55 MΩ/cm; Total hardness 198 mg/L; Chlorides 30 mg/L; and fluoride 0.9 mg/L) *ad libitum*.

Animals of group I and IV were sacrificed by cervical dislocation on day 91. Group III animals were, however, allowed to recover for 90 days under control conditions and were sacrificed on day 181, along with respective control animals (group VI), and are henceforth referred to as post-treatment and post-control groups, respectively.

**Phase 2-4**— Four young male mice (30-35 days old) of phase 2-4 received diet supplements (each @ 230 mg/kg body wt.) along with standard chow for 45 days prior to entry into experimental protocol (Phase 2: Spirulina, Phase 3: tamarind pulp, and Phase 4: Spirulina + tamarind pulp in ratio of 1:1). Thereafter, general layout of phase 2-4 experiments was similar to phase 1 i.e., 60 mice of each phase were divided in to 6 groups having 3 groups each of control & fluoride treatments, as detailed earlier. The fine suspension of spray-dried powder of *Spirulina platensis* (Sunova capsule, Dabur Ltd.) and semidried pulp of Tamarind fruits (ripe) were prepared separately in the distilled water and were administered orally through gavage (0.5 mL/day/mice) to Swiss albino mice.

Both control and fluoride treated mice reared only on standard chow (Phase 1) are referred to as standard feed group hereafter in the text while those reared on diet supplements + Standard chow (Phase 2-4) as Spirulina, tamarind and Spirulina + tamarind groups, respectively.

Animals were observed, at least twice a day, for clinical signs and symptoms of toxicity. Their feed and water consumption were recorded after every 24 hour, but body weight only at the termination of experiment.

**Autopsy**— All animals of Phase 1-4 were weighed prior to sacrifice at the termination of study. A midline abdominal incision was performed and blood was collected through cardiac puncture into the vials for serum biochemistry studies. Their kidneys were removed, cleaned, blotted free of blood and weighed.

**Biochemical analyses**— Urea, creatinine, total protein, acid (ACP) and alkaline phosphatase (ALP) content were estimated using diagnostic kits (Span Diagnostics Ltd., India). Protein (by Lowry’s method) and acid and alkaline phosphatase contents were analyzed in the tissue homogenate of kidney.

**Histological and Morphometric studies**— Kidney fixed in Bouin’s fluid were embedded in paraffin wax. Sections (6 μm thick) stained with hematoxylin-eosin were examined under light microscope. Morphometric studies included measurements of Bowman’s capsule, glomeruli, Bowman’s space, area
of proximal and distal convoluted tubules using oculometer standardized with stage micrometer. Further, cell counting in tubules and of podocyte in glomerular tuft were also made.

**Data analysis**

The data are expressed as mean ± the standard error of the mean. The differences among means were analyzed by one way ANOVA using a Systat 5.0 software program.

**Results and Discussion**

**Behavior and general health**

Fluoride exposed mice of standard feed and Spirulina groups had frequent fights. Such aggressive behavior was absent in other diet supplement groups (tamarind and Spirulina + tamarind groups) and also in control mice. The diet also affected fur quality. The control mice of Spirulina group had superior fur quality (more hairy and shiny) which was normal in other feeding groups. The fur was less hairy with poor shining in F-treated groups, except Spirulina group. Further, only treated mice of standard feed group (sub-acute) had black nails.

**Alterations in feed and water intake and related parameters**

Fluoride exposure decreased food intake (5-43%, Table 1). The maximum reduction in feed intake was found in Spirulina and tamarind groups of sub-acute exposure (31-43%) which was insignificant during their sub-chronic exposure (4-15%), and also in fluoride treatments of standard feed (6-16%) and Spirulina + tamarind groups (2-7%). Thus, diet supplements in combination were found to be more ameliorative in fluoride treatments.

Appetite loss possibly led to breakdown of cellular macromolecules to meet energy requirement that decreased body weights of fluoride exposed mice (10-18%, Table 1). In contrast to feed intake, maximum reduction in body weights were found in the standard feed groups. It is likely that preemptive feeding of diet supplements minimized toxic effects.

Table 1— Feed (g/animal) and water intake (mL/animal), body (g) and kidney weights (mg) of control, fluoride treated and post-treatment mice of standard feed and diet supplement groups; Significant (P) at *<0.05; **<0.01; ***<0.001.

<table>
<thead>
<tr>
<th>Feeding Groups</th>
<th>Feed intake</th>
<th>Body weight</th>
<th>Water intake</th>
<th>Kidney weight</th>
</tr>
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<tbody>
<tr>
<td>Standard feed groups</td>
<td>4.3±0.5 (410)</td>
<td>24.8±1.2** (118)</td>
<td>8.1±0.7 (11)</td>
<td>173.0±12.5 (15)</td>
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<tr>
<td>Fluoride treatments: Group-I</td>
<td>4.5±0.1-6.4 ±0.1 (6-9)</td>
<td>27.0±1.3 (11)</td>
<td>7.2±0.1-8.3 ±0.2 (76-24)</td>
<td>183.4±9.5 (11)</td>
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<tr>
<td>Group-II</td>
<td>4.7±0.1-5.6±0.3 (21-45)</td>
<td>34.3±1.2 (11)</td>
<td>6.3±0.3-8.4 ±0.1 (19-14)</td>
<td>268.0±22.0 (14)</td>
</tr>
<tr>
<td>Group-III</td>
<td>4.8±0.8</td>
<td>30.2±0.5</td>
<td>8.0±0.4</td>
<td>182.8±8.9</td>
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<tr>
<td>Controls: Group-IV</td>
<td>4.7±0.2-7.0±0.2</td>
<td>26.8±1.4</td>
<td>6.6±0.2-6.8±0.1</td>
<td>205.8±16.2</td>
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<td>Group-V</td>
<td>5.3±0.4-8.5±0.1</td>
<td>34.8±1.1</td>
<td>6.9±0.3-9.8±0.2</td>
<td>311.0±7.0</td>
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<tr>
<td>spirulina groups</td>
<td>3.4±0.6 (3)</td>
<td>31.8±0.5 (33)</td>
<td>6.4±0.3 (110)</td>
<td>180.4±17.6 (18)</td>
</tr>
<tr>
<td>Fluoride treatments: Group-I</td>
<td>5.1±0.2-5.8±0.2 (115-132)</td>
<td>31.6±13.3 (111)</td>
<td>6.3±0.2-8.0±0.3 (13-43)</td>
<td>287.6±15.4 (114)</td>
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<td>Group-II</td>
<td>5.6±0.9-6.8±0.2 (44-119)</td>
<td>31.0±0.6 (4)</td>
<td>7.8±0.1-10.1±0.2 (128-59)</td>
<td>269.1±6.6 (12)</td>
</tr>
<tr>
<td>Group-III</td>
<td>4.9±0.3</td>
<td>32.8±0.5</td>
<td>5.8±0.2</td>
<td>195.3±7.6</td>
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<tr>
<td>Controls: Group-IV</td>
<td>4.4±0.2-6.3±0.1</td>
<td>28.4±0.8</td>
<td>5.6±0.2-6.1±0.1</td>
<td>251.6±15.9</td>
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<td>Group-V</td>
<td>4.7±0.37-7.1±0.2</td>
<td>32.3±0.9</td>
<td>5.7±0.2-6.3±0.1</td>
<td>273.1±8.6</td>
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<td>Tamarind groups</td>
<td>2.3±0.3 * (1)</td>
<td>32.4±1.2 (17)</td>
<td>7.0±0.4** (133)</td>
<td>188.1±27.3 (112)</td>
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<td>Fluoride treatments: Group-I</td>
<td>4.5±0.1-7.0±0.1 (14-19)</td>
<td>30.8±0.5 (13)</td>
<td>7.1±0.1-8.0 ±0.7(16-18)</td>
<td>218.2±12.7 (15)</td>
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<td>Group-II</td>
<td>5.3±0.04-6.1±0.3 (13-139)</td>
<td>35.2±1.0 (11)</td>
<td>6.3±0.3-8.4 ±0.1 (121-31)</td>
<td>247.2±13.9 (13)</td>
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<td>Group-III</td>
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<td>31.2±0.8</td>
<td>5.3±0.2</td>
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<tr>
<td>Controls: Group-IV</td>
<td>4.7±0.1-6.4±0.1</td>
<td>29.8±0.8</td>
<td>6.7±0.1-6.7 ±0.2</td>
<td>228.4±4.6</td>
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<tr>
<td>Group-V</td>
<td>4.4±0.1-6.1±0.3</td>
<td>34.8±0.9</td>
<td>4.5±0.1-6.4 ±0.2</td>
<td>241.2±8.6</td>
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<tr>
<td>Group-VI</td>
<td>3.5±0.3 (3)</td>
<td>29.4±1.5 (15)</td>
<td>5.1±0.3 (12)</td>
<td>165.0±20.0 (115)</td>
</tr>
<tr>
<td>Spirulina + Tamarind groups</td>
<td>4.0±0.1-5.8 ±0.1 (7-12)</td>
<td>32.8±1.6 (13)</td>
<td>6.4±0.1-6.7 ±0.2 (15-6)</td>
<td>235.4±18.8 (12)</td>
</tr>
<tr>
<td>Fluoride treatments Group-I</td>
<td>4.0±0.1-5.8±0.2 (7-14)</td>
<td>31.3±0.4 (10)</td>
<td>4.4±0.2-5.8 ±0.1(17-28)</td>
<td>230.6±7.1 (110)</td>
</tr>
<tr>
<td>Group-II</td>
<td>3.6±0.4</td>
<td>30.8±0.5</td>
<td>5.2±0.1</td>
<td>172.8±8.0</td>
</tr>
<tr>
<td>Group-III</td>
<td>4.3±0.1-5.7±0.1</td>
<td>31.8±1.3</td>
<td>5.8±0.1-7.1±0.2</td>
<td>230.2±21.7</td>
</tr>
<tr>
<td>Group-IV</td>
<td>4.2±0.1-5.7±0.5</td>
<td>34.8±1.3</td>
<td>4.3±0.1-5.0±0.1</td>
<td>257.0±22.0</td>
</tr>
</tbody>
</table>

[Fluoride treatments: Group (G-I), sub-acute; G-II, sub-chronic; and G-III, post-treatment. Controls: G-IV, sub-acute; G-V, sub-chronic; and G-VI, post-control]
on feed intake of Spirulina and tamarind groups during sub-acute exposure.

Fluoride exposure, however, increased their water intake (10-40%, Table 1). Wallin and Kaplan\textsuperscript{24} reported that fluoride ion may render the collecting duct unresponsive to vasopressin or decrease vasopressin induced cAMP generation that reduces water reabsorption in Henley’s loop which is lost through increased urination (polyuria). The excess water loss is compensated by its higher intake (polydipsia). Both, feed intake and body weight of diet supplement groups, recovered during sub-chronic exposure and were almost similar to their controls (Table 1). Chinoy and Sharma\textsuperscript{25} also made similar findings in fluoride treated Wistar rats receiving vitamins as diet supplement.

Compared with controls, kidney weights decreased after sub-acute (5-12%) exposure of standard feed as well as diet supplement groups but only in standard feed groups (11-14%) of sub-chronic exposure and posttreatment (Table 1). Thus, diet supplements played protective role during sub-chronic exposure. Rao \textit{et al.}\textsuperscript{26} also reported protective role of melatonin on kidney weight of fluoride treated mice. The extensive lysis of soft tissue possibly decreased kidney weight in the present study [(Plate 1 (sub-acute) and 2 (sub-chronic) E)].

Plate 1—T.S. of kidney (cortex region) of control (A-D); and F– treated mice of sub-acute exposure (E-L)

Controls (400X): Fig. A (Standard feed); B (Spirulina); C (Tamarind); and D (Spirulina + Tamarind) showing normal structure of Bowman’s capsule (BC), Visceral (VL) and Parietal layer (PL), Podocyte (PD), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT), Brush border epithelium (BBE), and Cuboidal epithelium (CE)

Fluoride Treatments: Standard feed group: Fig. E (400X) showing Tissue fibrosis ( ), Lysis in glomeruli ( ), Atrophic glomeruli ( ), Dilation of convoluted tubules ( ), Degeneration of tubular epithelium ( ), Nuclear condensation ( ), Chromatin material aggregation close to nuclear membrane ( ), Fig. I. (1000X) showing Lysis in glomerulus ( ), Thickening of parietal and visceral layer of Bowman’s capsule ( ) and fusion of glomeruli with the parietal layer of Bowman’s capsule ( ), Necrosed ( ) and fused podocytes ( ), Necrotic part of glomerular tuft ( )

Spirulina group: Fig. F (400X) showing Dilation of PCT and DCT ( ), Thickening of parietal layer of Bowman’s capsule ( ), Chromatin material aggregation close to nuclear membrane ( ), Nuclear condensation ( ); Fig. J (1000X) showing Necrosed ( ) and fused podocytes ( ), Mesangial cell proliferation in glomerulus ( ), Thickening of parietal and visceral layer of Bowman’s capsule ( ) and fusion of glomeruli with the parietal layer of Bowman’s capsule ( ), Lysis in glomerulus ( ), Necrotic part of glomerular tuft ( ).

Tamarind group: Fig. G (400X) showing Thickening of parietal layer of Bowman’s capsule ( ), Glomerulus fusion with parietal layer of Bowman’s capsule ( ), Lysigenous cavity ( ); Fig. K (1000X) showing Thickening of parietal and visceral layer of Bowman’s capsule ( )

Spirulina+Tamarind group Fig. H (400X); Fig. L (1000X) showing Normal structure of Bowman’s capsule
Alterations in histopathology

Bowman’s capsule and tubules (PCT and DCT) found compactly arranged in the cortical region of control kidneys (Plate 1 and 2, A-D) had interstitial space with either bleeding or mononuclear cell infiltration or both in fluoride treatments (Plate 1 and 2, E-L). Lysigenous cavities were also present in standard feed group of sub-acute exposure. Chattopadhayay et al. reported interstitial bleeding in fluoride treated Swiss albino mice while mononuclear cell infiltration was observed in Wistar rats. Mononuclear cells encircled tubules (peritubular) and glomerulli (perivascular) in the treated mice of Spirulina group during sub-acute exposure. Karaoz et al. made similar finding in fluoride treated Wistar rats.

The cortical regions of treated mice of standard feed groups had regions with well developed tissue fibrosis (Plate 1 and 2 E). It was found absent in the controls and also in the diet supplement groups, except Spirulina (sub-acute exposure; Plate 1F) and Spirulina + tamarind group (sub-chronic exposure) (Plate 2H) having only poor fibrosis. Cellular infiltration and fibrosis in the cortex are

![Plate 2—T.S. of kidney (cortex region) of control (A-D); and F treated mice of sub-chronic exposure (E-L)](image-url)

Controls (400X): Fig. A (Standard feed), B (Spirulina), C (Tamarind) & D (Spirulina + Tamarind) showing normal structure of Bowman’s capsule (BC), Visceral (VL: Black thin arrow) and Parietal layer (PL: Blue thin arrow), Podocyte (PD: Green thin arrow), Proximal convoluted tubule (PCT), Distal convoluted tubule (DCT), Brush border epithelium (Red thick arrow) & Cuboidal epithelium (Yellow thick arrow)

Fluoride Treatments: Standard feed group: Fig. E (400X) showing Tissue fibrosis (▲▲), Dilation of convoluted tubules (▲), Cell debris in tubular lumen (●), Cellular vacuolization (▲▲▲), Nuclear condensation (▲▲▲▲); Fig. I (1000X) showing Prominent Lysis in Glomerular tuft (▲), Glomeruli attached with parietal layer of Bowman’s capsule (▲▲▲▲), Necrosed and shrunked podocytes (▲▲▲▲), fused podocytes (▲▲▲▲)

Spirulina group: Fig. F (400X) showing Glomeruli attached with the parietal layer of Bowman’s capsule (▲▲▲▲), Degeneration of the tubular epithelial cells and dead epithelium lining of the tubules sloughed in to the lumen (▲▲), Mesangial cell proliferation (▲▲▲▲), Fig. J (1000X) showing Glomeruli attached completely with Bowman’s capsule (▲▲▲▲), Fused podocytes (▲▲▲▲)

Tamarind group: Fig. G (400X) showing Mononuclear cell infiltration (▲▲), Thickening of parietal layer of Bowman’s capsule (▲▲▲▲), Enlargement of tubules (▲▲▲▲), Fig. K (400X) showing Thickening of parietal layer of Bowman’s capsule (▲▲▲▲), Mild lysis in glomerulus (▲▲▲▲), Enlargement of tubules (▲▲▲▲)

Spirulina + Tamarind group: Fig. H (400X) showing Poor tissue fibrosis (▲▲▲▲️), Fig. L (400X) Showing normal structure of Bowman’s capsule
common characteristics for virtually all progressive renal diseases with proteinuria. All such abnormalities were, however, found almost absent in the post-treated mice.

**Bowman’s capsule**— Fluoride exposure caused dilation (16-38%) of Bowman’s capsule in the standard feed groups. No such dilation was found in the diet modulated groups, except Spirulina group having little dilation (8%) during sub-chronic exposure (Fig. 1A). Zhan et al. made similar finding in fluoride treated pigs. Fluoride withdrawal led to recovery of the post-treated mice, particularly in Spirulina + tamarind group (Fig. 1A).

The short term fluoride exposure increased thickness of parietal and visceral layers of Bowman’s capsule in all feeding groups (Plate 1 E-G, I and J) but only of parietal layer in sub-chronic treatments (Plate 2 E-G and K), possibly on account of increased collagen synthesis. Parietal layer was also fused with glomeruli in all treatments of sub-acute exposure (Plate 1 G, I and J) but only in standard feed and Spirulina groups of sub-chronic exposure (Plate 2 F, I and J), and therefore, these were not centrally placed in the cavity (Plate 2F). The glomeruli in Spirulina + tamarind and tamarind group were, however, centrally placed (Plate 2 G, H, K and L). In post-treated mice, thickening of parietal layer and its fusion with glomeruli was found only in the standard feed group (Plate 1 I). Thus, a complete recovery was observed in the diet modulated groups.

Glomerulus size decreased (9%) during sub-acute exposure of standard feed group but followed an opposite trend (33%) in sub-chronic exposure (Fig. 1B). No such alterations were found in Spirulina groups while these were at lower magnitude (6-13%) in other groups of sub-chronic exposure (Fig. 1B). The shrinkage of glomeruli was possibly on account of degenerative changes while their dilation might be an indication to increase glomerulus filtration rate (GFR) to reduce fluoride burden. Fluoride withdrawal led to recovery of the post-treated mice, particularly of diet supplement groups (Fig. 1B).

The dilation of Bowman’s capsule but shrinkage of glomerulus increased Bowman’s space in fluoride

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**Fig. 1**— Diameter of (A) Bowman’s capsule; (B) glomeruli; and (C) Bowman’s space. [in µm of controls and fluoride treated mice in different feeding groups]. [Std, Standard feed; S, Spirulina; and T, Tamarind. Mean ± SEM, Significant (P) at *<0.05; **<0.01; and ***<0.001]
treatments (sub-acute 19-85%; sub-chronic 10-68%), especially in the standard feed groups (Fig. 1C). Bhatnagar et al.32 made similar findings in Labeo rohita. Bowman’s space was, however, similar to control mice in tamarin and Spirulina + tamarin groups of sub-acute exposure (Fig. 1C). Fluoride withdrawal led to shrinkage of Bowman’s capsule and its glomerulus in the post-treated mice, being maximum in standard feed group, moderate in Spirulina group and minimum in tamarin and Spirulina + tamarin group. Accordingly, the size of Bowman’s space varied (Fig. 1C).

The arterioles and adjacent part of the glomerular tuft were necrotic during sub-acute exposure (except Spirulina + tamarin group; Plate 1 I and J), and also in the standard feed group of sub-chronic and post-treated mice.

Fluoride exposure caused extensive lysis in the glomeruli of standard feed groups (Plate 1 and 2 E, I) but it was mild (Spirulina group Plate 1 J) to almost nil in the diet supplement groups (tamarin and Spirulina+ tamarin groups Plate 1 and 2 G, H, K and L). The extensive lysis led to sclerotisation of about 8% glomeruli of the standard feed group of sub-acute exposure. Often, these sclerotic regions become adherent to and disrupt Bowman’s capsule. As a result, glomeruli shift towards one side of Bowman’s capsule (Plate 1 E). Such lesions provide additional site for direct leakage of the glomerular ultrafiltration into the peritubular space, a mis-directional ultrafiltration as described by Kriz et al.33. Sclerotisation of glomeruli has so far not been reported after fluoride treatment. Kiran et al.34 observed glomerular sclerotic condition aggravated with increased duration of diabetes in streptozotocin-induced diabetic rats. Singh et al.35 reported sclerotisation/glomerulosclerosis in Swiss albino mice treated with cadmium chloride and found curcumin to check its nephrotoxic effects.

Podocytes along with mesangial cells maintain structural integrity of the glomerulus. Fluoride exposure decreased podocyte counts (<15/glomerulus) that was maximum in standard feed groups (24-58%). Hypocellularity was, however, meager in the diet supplement groups (4-8%) and also in all post-treated mice. It might be due to necrosis of most of the podocytes present as darkly stained body in glomerulus (Plate 1 and 2 I) while a few of them fused forming a large binucleated distinct structure in all treated groups (Plate 1 and 2 I). The reduction in podocytes number/glomerulus (podocytopenia) is an indicator of progressive renal disease36 and this may exacerbate the development of proteinuria37.

Mesangial cells proliferated at the glomerulus base in all feeding groups of sub-acute exposure (Plate 1J); standard feed and Spirulina groups of sub-chronic exposure (Plate 2F) and post-treated mice of standard feed group. Being phagocytic in nature, mesangial cells were possibly associated with cleaning of cell debris formed due to necrosis of podocytes and lysis of capillaries in glomeruli38. Their absence in tamarin and Spirulina + tamarin groups might be attributed to lack of cell debris in them.

**Tubular alteration**— Fluoride exposure led to enlargement of proximal (9-107%) and distal (18-93%) tubules. The maximum enlargement in proximal tubule occurred during sub-acute exposure while that of distal tubule in sub-chronic exposure. Among different feeding groups, maximum tubular enlargement was found in the standard feed groups (Sub-acute: PCT 107%, DCT 82%; Sub-chronic: PCT 24%, DCT 93%) that was relatively lower in the diet supplement groups (Sub-acute: PCT 10-52%, DCT 18-46%; Sub-chronic: PCT 9-36%, DCT 34-65%; Fig. 2 A and B). Karaoz et al.28 also reported dilation in both proximal and distal tubules in Wistar albino rat. Both PCT (8-32%) and DCT (12-37%) were also enlarged in the post-treated mice but their percentage increase was lesser in comparison to sub-chronic exposure suggesting recovery of tubules following fluoride withdrawal, particularly in the Spirulina + tamarin group (Fig. 2 A and B).

Compared with controls, epithelial cell counts decreased in the tubules after fluoride exposure (PCT 5-7%, DCT 10-16%), especially in standard feed groups. Their counts recovered in the post-treated mice but at a faster pace in the diet modulated groups (Fig. 3A andB). The reduction in epithelial cell counts was on account of their release from the tubular wall into lumen where they were present in necrosed/disintegrated state, as reported by other workers in fluoride treated Swiss albino mice37 and Wistar albino rats28. Such toxic effects reduce reabsorption of essential metabolites and ions from filtrate.

Standard feed and tamarin groups of sub-acute fluoride exposure had nuclear aberrations in the epithelial cells of tubules (Plate 1 E, G). The nucleus shrunk and stained dark homogenously (pyknotic nucleus). Chromatin material in few nuclei aggregated close to nuclear membrane (Plate 1 E). Zhan et al.39...
also reported nuclear condensation in tubular epithelial cell of fluoride treated pigs. Whereas Susan et al.\textsuperscript{31} reported margination and condensation of chromatin in epithelial cells of DCT in methyl mercury treated Pekin ducks.

Present study has revealed protective role of diet supplements on histopathology of kidney, particularly in combination, possibly on account of their antioxidant contents\textsuperscript{10,18}.

**Tissue (kidney) and Serum biochemistry**

*Protein*— Sub-acute fluoride exposure decreased (7-57\%) protein contents in both tissue and serum while its values increased (22-128\%) after sub-chronic exposure and also in post-treatments (Fig. 4 A and B).

By increasing generation of free radicals and lipid peroxidation and counteracting antioxidant defense mechanism, fluoride shifts the oxidant/antioxidant balance towards oxidative stress that damage soft tissue and biomacromolecules such as proteins and nucleic acids as noted during short-term exposure to higher fluoride concentration. The exposure to lower concentration for longer period, however, led to synthesis of stress protein, such as Hsp 70, a heat shock protein\textsuperscript{27} imparting protection to cell

![Area](image)

**Fig. 2**—Area of (A) PCT; and (B) DCT. [In \(\mu\text{m}^2\) of controls and fluoride treated mice in different feeding groups]. [Std, Standard feed; S, Spirulina; and T, Tamarind. Mean = SEM, Significant \((P)\) at *\(<0.05\); **\(<0.01\); and ***\(<0.001\)]

![Cell counts](image)

**Fig. 3**—Cell counts in (A) PCT; and (B) DCT. [In cells/tubule of controls and fluoride treated mice in different feeding groups]. [Std, Standard feed; S, Spirulina; and T, Tamarind. Mean = SEM, Significant \((P)\) at *\(<0.05\); **\(<0.01\); and ***\(<0.001\)]
organelles. Thus, treated mice possibly adapted to long term fluoride exposure.

Acid phosphatase (ACP)—ACP is an inducible enzyme because its activity goes up in response to toxic effects that enzyme counteracts. Its values also increased in the diet supplement controls compared with standard feed controls indicating its increased activity in the experimental animals fed with antioxidant rich diet supplements, and thereby improve their health as observed in the present study (Fig. 5 A and B).

Compared with controls, fluoride exposure increased (10-22%) ACP activities in kidney and serum of standard feed groups while an opposite trend (29-73%) was observed in the diet supplement groups, except Spirulina and Tamarind groups that showed a little increase (6-22%, Fig. 5 A and B).

Based on our findings on control mice, we suggest that standard feed groups having lesser ACP activities require more ACP to counteract Fluoride (F⁻) toxicity. Spirulina and tamarind groups which were having sufficient ACP activities to counteract toxic effect of F⁻.
did not respond similar to standard feed groups following F exposure. This explains why ACP activities increased in standard feed groups but declined in Spirulina and Tamarind groups. A further rise in ACP activities of treated mice of Spirulina + tamarind groups, however, led maximum recovery among diet modulated groups, as evident from histopathological findings (Plate 1 and 2 D,H, L).

Alkaline phosphatase (ALP)— ALP activities which showed little increase in the kidney of treated mice of Spirulina and Spirulina+tamarind groups (10 - 21%) during their sub-acute exposure, however, increased markedly in standard feed group (83%) of sub-chronic exposure (Fig. 6A). However, it differed little with controls in the treated mice of tamarind groups and decreased (13-28%) in the diet supplemented groups during sub-chronic exposure.

Short term treatment with ALP restored renal function in patients with sub-acute kidney injury suggesting renoprotective role of ALP, and therefore, an increase in ALP levels in diet supplements during sub-acute exposure suggests their renoprotective role. Similar increase in its level in standard feed group of sub-chronic exposure could be attributed to response of immune system to counteract F toxicity following prolonged exposure, since it is also an inducible enzyme similar to ACP. It is likely that F toxicity in the diet supplement groups of sub-chronic exposure subsided earlier which led to lowering its levels in them.

Compared with controls, ALP levels increased in the serum of treated mice though at a slower pace in the standard feed groups (15-21%) when compared with diet modulated groups (28-65%, Fig. 6B). The diet supplement groups thus, responded better to counteract F toxicity. ALP activity is localized in the brush border of the PCT of kidney and increase in its activity at a slower pace in the standard feed groups may be related to higher structural aberrations in the kidney compared with diet modulated groups.

Interestingly, ALP activities in tissue (418-570 µmol/mg) and serum (136-344 IU/L) of diet modulated controls were found higher than that of the standard feed controls (Tissue: 257- 459 µmol/mg, Serum: 96-234 IU/L) of sub-acute and sub-chronic exposure. This suggests that diet modulated controls were equipped better to deal with pollutants such as fluoride.

Urea and Creatinine

Compared with standard feed controls, urea and creatinine levels increased in the diet modulated controls (Fig. 7 A and B). Fluoride exposure increased serum urea and creatinine levels in standard feed groups which were either similar or less than controls in the diet supplement groups.

Fluoride exposure increased serum urea and creatinine levels in pigs and rabbits but only urea in Sprague–Dawley rats. However, diet supplements tamarind fruit pulp and Moringa oleifera seeds have been reported to have ameliorating effect on urea and creatinine contents in fluoride treated rabbits. Salazar et al. reported haemodilution in Swiss albino mice reared on diet supplement Spirulina. Serum albumin is known to increase in response to blood
dilution to balance osmotic pressure. However, it is a large colloidal protein that may impede glomerular filtration rate (GFR), and thereby reduce loss of excretory products such as urea and creatinine. This explains why urea and creatinine content increased in diet modulated controls in comparison to standard feed controls.

A comparison of urea and creatinine contents in fluoride treated mice of sub-chronic exposure revealed their higher levels in standard feed group (urea 76 mg/dl, creatinine 1.68 mg/dl) compared to diet supplement groups [urea 45-55 mg/dl, creatinine 0.54-1.2 mg/dl, (Fig. 7 A and B)]. This would mean better efficiency of kidney in the diet supplement groups owing to their better structural organization as observed in histopathological studies above.

Various biochemical parameters of tissue and serum in the post-treated mice differed little with post-controls, particularly in the supplement groups, suggesting faster recovery compared to the standard feed group.

Present study has established toxic effects of fluoride on general health of Swiss albino mice and their kidney. The intensity of fluoride toxicity, however, decreased in diet supplemented groups, especially when administered in combination.

Acknowledgements
Author NY gratefully acknowledges CSIR; SS, DST (LS194/WOS-A/2010); and AP, ICMR for award of fellowships.

References


