

## BENEFICIAL EFFECTS OF A PROTEIN RICH DIET ON FLUORIDE INDUCED FREE RADICAL TOXICITY IN THE LIVER OF MALE MICE

NJ Chinoy,<sup>a</sup> D Mehta, DD Jhala

Ahmedabad, India

**SUMMARY:** The effects on free radical toxicity in the liver of male mice from their ingestion of protein enriched and protein deficient diets along with 5, 10, and 20 mg NaF/kg body wt were investigated. Feeding a protein deficient diet with any of the three NaF doses for 30 days resulted in a significant decrease in levels of glutathione and reduced ascorbic acid as well as activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase in the liver. On the other hand, significant increases in lipid peroxides (LPO), dehydroascorbic acid (DHA), and fluoride levels were observed. The increase in total ascorbic acid (TAA) levels was less significant. These changes also occurred in mice fed the standard control protein diet plus the same doses of NaF. By contrast, when mice were fed a protein enriched diet along with NaF, none of the liver parameters studied were affected but were essentially the same as in mice fed the control protein diet without added fluoride. These studies indicate that dietary protein supplementation has a beneficial effect on liver function and is conducive to recovery from fluoride toxicity. The results also indicate the importance of adequate protein in the diet especially in developing countries where dietary protein deficiency and malnutrition are often present.

Keywords: Free radical toxicity; Liver enzymes; Mice liver and fluoride; Protein deficient diet; Protein enriched diet.

### INTRODUCTION

In biological systems, free radicals are highly reactive species that have an unpaired electron, e.g., the hydroxyl ( $\cdot\text{OH}$ ) and superoxide anion ( $\cdot\text{O}_2^-$ ) radicals, which can cause cellular damage. On record are various free radical toxicity manifestations in liver caused by fluoride as reflected in structural alterations, apoptosis, oxidative stress, etc., that affect liver function.<sup>1-9</sup> However, the specific effects of protein supplemented and protein deficient diets on fluoride induced free radical toxicity in the liver of male mice have not been adequately explored. Therefore, the present study was undertaken.

### MATERIALS AND METHODS

*Animals:* Healthy, adult male mice (*Mus musculus*) of Swiss strain weighing between 25 and 35 g were obtained from Cadila Pharmaceuticals, Ghodasar, Ahmedabad, India, under the Animal Maintenance and Registration No. 167/1999/CPCSEA from the Ministry of Social Justice and Empowerment, Government of India and the Committee for the Purpose of Control and Supervision of Experiments on Animals, Chennai, India. The mice were housed in an air-conditioned animal unit at  $26\pm 2^\circ\text{C}$  with a 10–12 hr of daylight/day. They were fed standard chow and given drinking water containing 0.6–1.0 ppm F available *ad libitum*.

*Exposure:* The mice were divided in to twelve groups as shown in the Experimental Protocol.

---

<sup>a</sup>For correspondence: Reproductive Endocrinology and Toxicology Unit, Department of Zoology, School of Sciences, Gujarat University, Ahmedabad-380 009, India.  
E-mail: zooldeptgu@satyam.net.in

## Experimental Protocol

| Group | Treatment and dose<br>(10-15 animals used in each group) | Duration<br>(days) | Day of autopsy |
|-------|--|--------------------|----------------|
| I     | Control protein diet (20% protein)                       | -                  | *              |
| II    | Protein deficient diet (5% protein)                      | 30                 | 31             |
| III   | Protein enriched diet (40% protein)                      | 30                 | 31             |
| IV    | Control protein diet + NaF (5 mg/kg body wt)             | 30                 | 31             |
| V     | Protein deficient diet + NaF (5 mg/kg body wt)           | 30                 | 31             |
| VI    | Protein enriched diet + NaF (5 mg/kg body wt)            | 30                 | 31             |
| VII   | Control protein diet + NaF (10 mg/kg body wt)            | 30                 | 31             |
| VIII  | Protein deficient diet + NaF (10 mg/kg body wt)          | 30                 | 31             |
| IX    | Protein enriched diet + NaF (10 mg/kg body wt)           | 30                 | 31             |
| X     | Control protein diet + NaF (20 mg/kg body wt)            | 30                 | 31             |
| XI    | Protein deficient diet + NaF (20 mg/kg body wt)          | 30                 | 31             |
| XII   | Protein enriched diet + NaF (20 mg/kg body wt)           | 30                 | 31             |

\*Sacrificed with treated groups.

Sodium fluoride (Loba Chemie, Bombay, 99% purity) was administered to mice orally using a feeding tube attached to a hypodermic syringe. The NaF was used at doses of 5, 10, and 20 mg/kg body wt. The dose was based on the oral LD<sub>50</sub> value of fluoride, which is 54.4 mg F/kg body wt in male mice.<sup>10</sup> Oral administration was preferred since water is the main source of fluoride among human populations in endemic areas.

*Diets:* The control protein diet, the protein enriched, and the protein deficient diets (corresponding to the amount of casein present) were prepared according to the protocol of the National Institute of Occupational Health (NIOH), Ahmedabad, India. The diets had the following composition:

| Diet              | Protein (%) | Casein (%) | Starch powder (%) | Salt mixture (%) | Vitamin mixture (%) | Ground nut oil (%) |
|-------------------|-------------|------------|-------------------|------------------|---------------------|--------------------|
| Control           | 20          | 23.53      | 63.47             | 4                | 2                   | 7                  |
| Protein deficient | 5           | 5.88       | 81.12             | 4                | 2                   | 7                  |
| Protein enriched  | 40          | 47.06      | 39.94             | 4                | 2                   | 7                  |

*Data collection:* The control and treated groups of animals were weighed on an animal weighing balance (Ohaus, USA), sacrificed by cervical dislocation after the respective treatments and the liver was dissected out carefully, blotted free of blood, and used for the study.

*Biochemical study:* The enzyme activities of superoxide dismutase (SOD) (E.C.1.15.1.1),<sup>11</sup> Catalase (CAT) (E.C.1.11.1.6),<sup>12</sup> and glutathione peroxidase (GSH-Px) (E.C.1.11.1.9)<sup>13</sup> were assayed in the liver by the methods cited. Similarly, the levels of lipid peroxide (LPO),<sup>14</sup> glutathione (GSH),<sup>15</sup> and total, dehydro and reduced ascorbic acid (TAA, DHA, and RAA)<sup>16</sup> were determined according to the methods cited. The fluoride levels in liver were estimated by an Orion Ion Analyser Orion, model 920A, and a fluoride ion selective electrode.

*Statistics:* For all biochemical parameters, a minimum of five or six replicates were assayed, and the data were subjected to statistical analysis by Student's t test and ANOVA.

## RESULTS

As seen in Tables 1–3, the enzyme activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), together with levels of glutathione (GSH) and reduced ascorbic acid (RAA) in the liver, were reduced significantly in Groups IV, VII, and X (Control protein diet + 5, 10, and 20 mg NaF/kg body wt) as compared to the control Group I mice without fluoride.

**Table 1.** Superoxide dismutase (SOD) (units/mg protein) and lipid peroxide (LPO) levels (nanomoles of MDA/mg tissue wt/60 min) in liver of Groups I–XII mice<sup>a</sup>

| Group | Treatment                                       | SOD                       | LPO                        |
|-------|---|---------------------------|----------------------------|
| I     | Control protein diet (20% protein)              | .57 ± 0.001               | 117.84 ± 4.96              |
| II    | Protein deficient diet (5% protein)             | 0.38 ± 0.010 <sup>‡</sup> | 161.42 ± 1.21 <sup>‡</sup> |
| III   | Protein enriched diet (40% protein)             | 0.57 ± 0.010              | 118.83 ± 1.42              |
| IV    | Control protein diet + NaF (5 mg/kg body wt)    | 0.36 ± 0.100 <sup>‡</sup> | 160.40 ± 1.66 <sup>‡</sup> |
| V     | Protein deficient diet + NaF (5 mg/kg body wt)  | 0.27 ± 0.005 <sup>§</sup> | 182.12 ± 0.98 <sup>§</sup> |
| VI    | Protein enriched diet + NaF (5 mg/kg body wt)   | 0.57 ± 0.004              | 119.08 ± 0.71              |
| VII   | Control protein diet + NaF (10 mg/kg body wt)   | 0.26 ± 0.005 <sup>§</sup> | 185.74 ± 0.67 <sup>§</sup> |
| VIII  | Protein deficient diet + NaF (10 mg/kg body wt) | 0.20 ± 0.006 <sup>§</sup> | 191.35 ± 1.25 <sup>§</sup> |
| IX    | Protein enriched diet + NaF (10 mg/kg body wt)  | 0.57 ± 0.008              | 118.62 ± 0.66              |
| X     | Control protein diet + NaF (20 mg/kg body wt)   | 0.20 ± 0.004 <sup>§</sup> | 199.28 ± 0.36 <sup>§</sup> |
| XI    | Protein deficient diet + NaF (20 mg/kg body wt) | 0.16 ± 0.010 <sup>§</sup> | 199.94 ± 0.73 <sup>§</sup> |
| XII   | Protein enriched diet + NaF (20 mg/kg body wt)  | 0.57 ± 0.002              | 118.78 ± 0.45              |

<sup>a</sup>Data are expressed as mean ± SE; \*P<0.05; †P<0.02; ‡P<0.01; §P<0.001; No sign = not significant.

Comparisons between: Group I with Groups II or III or IV or VII or X individually; Group II with Groups V or VIII or XI individually; Group III with Groups VI or IX or XII individually.

**Table 2.** Glutathione (GSH) ( $\mu\text{g}/100$  mg fresh tissue wt), catalase (CAT) (units/min/mg protein) and glutathione peroxidase (GSH-Px) (nanomoles of NADPH oxidized/min/mg protein) activity in liver of Groups I–XII mice<sup>a</sup>

| Group | Treatment                                       | GSH                           | CAT                           | GSH-PX                        |
|-------|---|-------------------------------|-------------------------------|-------------------------------|
| I     | Control protein diet (20% protein)              | 82.17 $\pm$ 0.68              | 60.70 $\pm$ 0.46              | 11.07 $\pm$ 0.25              |
| II    | Protein deficient diet (5% protein)             | 61.38 $\pm$ 1.07 <sup>§</sup> | 47.63 $\pm$ 0.51 <sup>‡</sup> | 08.22 $\pm$ 0.50 <sup>§</sup> |
| III   | Protein enriched diet (40% protein)             | 82.51 $\pm$ 0.19              | 60.63 $\pm$ 0.49              | 11.05 $\pm$ 0.26              |
| IV    | Control protein diet + NaF (5 mg/kg body wt)    | 56.97 $\pm$ 0.88 <sup>§</sup> | 45.65 $\pm$ 0.63 <sup>‡</sup> | 08.45 $\pm$ 0.33 <sup>§</sup> |
| V     | Protein deficient diet + NaF (5 mg/kg body wt)  | 53.89 $\pm$ 0.95 <sup>‡</sup> | 38.96 $\pm$ 0.42 <sup>§</sup> | 07.74 $\pm$ 0.35              |
| VI    | Protein enriched diet + NaF (5 mg/kg body wt)   | 81.95 $\pm$ 0.56              | 60.45 $\pm$ 0.44              | 11.08 $\pm$ 0.21              |
| VII   | Control protein diet + NaF (10 mg/kg body wt)   | 47.43 $\pm$ 1.25 <sup>§</sup> | 31.72 $\pm$ 0.52 <sup>§</sup> | 07.60 $\pm$ 0.10 <sup>§</sup> |
| VIII  | Protein deficient diet + NaF (10 mg/kg body wt) | 41.14 $\pm$ 1.32 <sup>§</sup> | 25.75 $\pm$ 0.62 <sup>‡</sup> | 06.90 $\pm$ 0.25 <sup>*</sup> |
| IX    | Protein enriched diet + NaF (10 mg/kg body wt)  | 81.84 $\pm$ 0.82              | 60.36 $\pm$ 0.29              | 11.04 $\pm$ 0.26              |
| X     | Control protein diet + NaF (20 mg/kg body wt)   | 31.33 $\pm$ 1.01 <sup>§</sup> | 25.11 $\pm$ 0.43 <sup>§</sup> | 06.89 $\pm$ 0.27 <sup>§</sup> |
| XI    | Protein deficient diet + NaF (20 mg/kg body wt) | 28.03 $\pm$ 0.75 <sup>§</sup> | 20.31 $\pm$ 0.54 <sup>§</sup> | 06.43 $\pm$ 0.17 <sup>§</sup> |
| XII   | Protein enriched diet + NaF (20 mg/kg body wt)  | 81.87 $\pm$ 0.54              | 60.43 $\pm$ 0.23              | 10.96 $\pm$ 0.24              |

<sup>a</sup>Data are expressed as mean  $\pm$  SE; \*P<0.05; †P<0.02; ‡P<0.01; §P<0.001; No sign = not significant.

Comparisons between: Group I with Groups II or III or IV or VII or X individually; Group II with Groups V or VIII or XI individually; Group III with Groups VI or IX or XII individually.

RAA was the least sensitive to treatment. A similar significant decrease occurred in Groups II, V, VIII, and XI (Protein deficient diet alone and with the three NaF doses). In these groups, SOD, CAT, and GSH appeared to be more sensitive to the treatment than GSH-Px and RAA.

The concentrations of total and dehydro ascorbic acid (TAA and DHA) as well as lipid peroxides (LPO) and fluoride were significantly enhanced in the liver of Groups IV, VII, and X. Similarly, LPO and fluoride were significantly increased in Groups II, V, VIII, and XI, but DHA and TAA were not significantly affected in Groups V, VIII, and XI (Tables 1–4).

In Groups III, VI, IX, and XII fed a protein enriched diet alone and with the three doses of NaF, no changes were observed in any of the parameters studied in comparison to the Group I control (Tables 1–4).

## DISCUSSION

In an earlier study<sup>17</sup> the toxic effects of fluoride on the reproductive organs of male mice were enhanced when NaF was administered with a protein deficient diet, whereas feeding a protein enriched diet had a beneficial influence in reducing fluoride induced toxicity in testis, cauda epididymis, and vas deferens of mice.

**Table 3.** Total, dehydro and reduced ascorbic acid (TAA, DHA and RAA) (mg/g fresh tissue wt) levels in liver of Groups I-XII mice<sup>a</sup>

| Group | Treatment                                       | TAA                      | DHA                      | RAA                      |
|-------|---|--------------------------|--------------------------|--------------------------|
| I     | Control protein diet (20% protein)              | 2.15 ± 0.43              | 1.33 ± 0.16              | 0.82 ± 0.24              |
| II    | Protein deficient diet (5% protein)             | 1.92 ± 0.19*             | 1.65 ± 0.34*             | 0.27 ± 0.03 <sup>‡</sup> |
| III   | Protein enriched diet (40% protein)             | 2.16 ± 0.08              | 1.34 ± 0.24              | 0.82 ± 0.12              |
| IV    | Control protein diet + NaF (5 mg/kg body wt)    | 2.32 ± 0.09*             | 1.76 ± 0.23*             | 0.56 ± 0.02*             |
| V     | Protein deficient diet + NaF (5 mg/kg body wt)  | 1.89 ± 0.14              | 1.69 ± 0.46              | 0.20 ± 0.03 <sup>‡</sup> |
| VI    | Protein enriched diet + NaF (5 mg/kg body wt)   | 2.15 ± 0.18              | 1.33 ± 0.24              | 0.82 ± 0.18              |
| VII   | Control protein diet + NaF (10 mg/kg body wt)   | 2.53 ± 0.32*             | 1.96 ± 0.19*             | 0.57 ± 0.13*             |
| VIII  | Protein deficient diet + NaF (10 mg/kg body wt) | 1.89 ± 0.15              | 1.77 ± 0.36              | 0.12 ± 0.01 <sup>‡</sup> |
| IX    | Protein enriched diet + NaF (10 mg/kg body wt)  | 2.12 ± 0.25              | 1.32 ± 0.25              | 0.80 ± 0.05              |
| X     | Control protein diet + NaF (20 mg/kg body wt)   | 2.61 ± 0.41 <sup>‡</sup> | 2.02 ± 0.36 <sup>‡</sup> | 0.59 ± 0.15*             |
| XI    | Protein deficient diet + NaF (20 mg/kg body wt) | 1.84 ± 0.34              | 1.79 ± 0.34              | 0.05 ± 0.01 <sup>‡</sup> |
| XII   | Protein enriched diet + NaF (20 mg/kg body wt)  | 2.03 ± 0.26              | 1.30 ± 0.46              | 0.73 ± 0.21              |

<sup>a</sup>Data are expressed as mean ± SE; \*P<0.05; †P<0.02; ‡P<0.01; §P<0.001; No sign = not significant. Comparisons between: Group I with Groups II or III or IV or VII or X individually; Group II with Groups V or VIII or XI individually; Group III with Groups VI or IX or XII individually.

The present data confirmed the occurrence of oxidative stress in the liver of mice fed a protein deficient diet with NaF resulting in structural and functional alterations in liver.<sup>2-9</sup> Similar results occur from NaF in the ovary<sup>18</sup> and the testis<sup>19</sup> of mice and in sperm suspensions of rats.<sup>20</sup> Moreover, NaF treatment caused a significant decrease in phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and glutathione in testis and cauda epididymis of adult male albino rats.<sup>21</sup> However, vitamin C and calcium were able to restore normalcy. These results indicate changes in cell membrane structure caused by fluoride. Sun et al.<sup>22</sup> also reported increased levels of lipid peroxides in liver, kidney, and testis together with a decrease in GSH-Px and SOD activities in fluorotic mice. The accumulation of free radicals and peroxides causing cell damage have also been found in people living in areas of endemic fluorosis.<sup>23</sup> In agreement with these findings, epidemiological studies of patients with dental and skeletal fluorosis in fluoride endemic regions of China also revealed an inhibition of GSH-Px and SOD activities in blood with increased lipid peroxide levels in serum.<sup>24</sup>

In monkeys with experimentally produced fluorosis, administration of a low protein diet caused accelerated development of bone fragility and a higher incidence of rarefaction.<sup>27</sup> Earlier studies<sup>25,26</sup> revealed that the amino acids glycine and/or glutamine were beneficial in promoting recovery from fluoride induced toxicity.

**Table 4.** Fluoride levels (ppm) in liver of Groups I-XII mice.<sup>a</sup>

| Group | Treatment                                       | Fluoride                     |
|-------|---|------------------------------|
| I     | Control protein diet (20% protein)              | 0.0061 ± 0.0005              |
| II    | Protein deficient diet (5% protein)             | 0.0061 ± 0.0005              |
| III   | Protein enriched diet (40% protein)             | 0.0060 ± 0.0005              |
| IV    | Control protein diet + NaF (5 mg/kg body wt)    | 0.0200 ± 0.0007 <sup>§</sup> |
| V     | Protein deficient diet + NaF (5 mg/kg body wt)  | 0.0210 ± 0.0007 <sup>§</sup> |
| VI    | Protein enriched diet + NaF (5 mg/kg body wt)   | 0.0060 ± 0.0005              |
| VII   | Control protein diet + NaF (10 mg/kg body wt)   | 0.0320 ± 0.0008 <sup>§</sup> |
| VIII  | Protein deficient diet + NaF (10 mg/kg body wt) | 0.0320 ± 0.0008 <sup>§</sup> |
| IX    | Protein enriched diet + NaF (10 mg/kg body wt)  | 0.0080 ± 0.0004              |
| X     | Control protein diet + NaF (20 mg/kg body wt)   | 0.0440 ± 0.0005 <sup>§</sup> |
| XI    | Protein deficient diet + NaF (20 mg/kg body wt) | 0.0520 ± 0.0007 <sup>§</sup> |
| XII   | Protein enriched diet + NaF (20 mg/kg body wt)  | 0.0080 ± 0.0003              |

<sup>a</sup>Data are expressed as mean ± SE; \*P<0.05; †P<0.02; ‡P<0.01; §P<0.001; No sign = not significant.

Comparisons between: Group I with Groups II or III or IV or VII or X individually; Group II with Groups V or VIII or XI individually; Group III with Groups VI or IX or XII individually.

Furthermore, protein supplementation was beneficial in overcoming the toxic effects of fluoride on testicular steroidogenesis, protein synthesis, carbohydrate metabolism, energy production, and oxidative metabolism in the various reproductive organs of male mice.<sup>17</sup> The results of the present study corroborate and are almost identical with those obtained for testis in an earlier paper,<sup>28</sup> and they indicate that the experimental conditions used cause similar changes in different organs of mice, but the severity of effects depends on the sensitivity of the organ *per se*. The results also suggest that protein supplemented diets might substantially mitigate certain fluoride induced health hazards in populations living in endemic areas.

Table 5. ANOVA of various parameters of mice liver

| Parameter      | Source of variation | SS       | df | MS       | F-cal    | F-tab    |
|----------------|---------------------|----------|----|----------|----------|----------|
| LPO            | Between Groups      | 68920.71 | 11 | 6265.519 | 1400.949 | 1.994579 |
|                | Within Groups       | 214.6723 | 48 | 4.472339 |          |          |
| TAA            | Between Groups      | 3.555965 | 11 | 0.32327  | 452.0831 | 1.994579 |
|                | Within Groups       | 0.034323 | 48 | 0.000715 |          |          |
| DHA            | Between Groups      | 6.444157 | 11 | 0.585832 | 3.458474 | 1.994579 |
|                | Within Groups       | 8.130742 | 48 | 0.16939  |          |          |
| RAA            | Between Groups      | 20.68106 | 11 | 1.880096 | 3.648935 | 1.994579 |
|                | Within Groups       | 38.80896 | 48 | 1.016853 |          |          |
| GSH-Px         | Between Groups      | 23885.42 | 11 | 2171.402 | 2687.259 | 1.994579 |
|                | Within Groups       | 38.78572 | 48 | 0.080036 |          |          |
| GSH            | Between Groups      | 1080.681 | 11 | 98.24374 | 1.44506  | 1.994579 |
|                | Within Groups       | 4120.294 | 48 | 85.83945 |          |          |
| SOD            | Between Groups      | 1.608118 | 11 | 0.146193 | 2226.282 | 1.994579 |
|                | Within Groups       | 0.003152 | 48 | 6.5705   |          |          |
| CAT            | Between Groups      | 14579.99 | 11 | 1325.454 | 5063.075 | 1.994579 |
|                | Within Groups       | 12.56584 | 48 | 0.261788 |          |          |
| Fluoride level | Between Groups      | 0.000142 | 11 | 1.2905   | 32.22348 | 1.994579 |
|                | Within Groups       | 1.9205   | 48 | 4.07     |          |          |

SS=Sum of squares; df=degrees of freedom; MS=Mean sum of squares;  
F-cal=Fisher calculated; F-tab=Fisher tabulated.

This work was presented at the XXIIIrd Conference of the International Society for Fluoride Research, Szczecin, Poland, June 11–14, 2000.

#### ACKNOWLEDGEMENT

The authors gratefully acknowledge the help from the Director, National Institute of Occupational Health (NIOH), Ahmedabad, India, for supplying the protocols for preparing the special diets used in this study.

#### REFERENCES

- 1 Czerny B, Put A, Mysliwiec Z, Juzyszyn Z. The influence of Quercetin on some parameters of lipid metabolism in rats chronically exposed to ammonium fluoride. *Fluoride* 2000;33(1):27-32.

- 2 Karimov K, Inoiatova FK, Inoiatov FSH. Toxic effects of various water pollutants on structural and functional parameters of hepatocytes. *Vopr Med Khim* 2002;48(2):174-9. [in Russian]. (Abstract in *Fluoride* 2003;36(1):61.)
- 3 Wang A, Xia T, Ran P, Bai Y, Yang K, Chen X. Effects of selenium and fluoride on apoptosis and lipid peroxidation in human hepatocytes. *Zhonzhua Yu Fang Yi Xue Za Zhi* 2002;36(4):235-8. [in Chinese]. (Abstract in *Fluoride* 2003;36(1):45-6.)
- 4 Shivashankara AR, Shivarajashankara YM, Bhat PG, Rao HS. Lipid peroxidation and antioxidant defense systems in liver of rats in chronic fluoride toxicity. *Bull Environ Contam Toxicol* 2002;64(4):612-6.
- 5 Chinoy NJ. Studies on fluoride, aluminium and arsenic toxicity in mammals and amelioration by some antidotes. In: Tripathi G, editor. *Modern trends in experimental biology*. New Delhi: CBS Publisher; 2002. p. 164-93.
- 6 Guo X-Y, Sun G-F, Sun Y-C. Oxidative stress from fluoride induced hepatotoxicity in rats. *Fluoride* 2003;36(1):25-9.
- 7 Chinoy NJ. Fluoride in the Environment. In: Chlubek D. editor. *Fluoride in medicine, biology and toxicology*. Warsaw, Poland: Katedra i Zaklad Biochemii i Chemii Pomorskiej Akademii Medycznej; 2003. p. 5-33.
- 8 Inkielewicz I, Krechniak J. Fluoride effects on glutathione peroxidase and lipid peroxidation in rats. *Fluoride* 2004; 37(1):7-12.
- 9 Chinoy NJ, Sharma AK, Patel TN, Memon R, Jhala DD. Recovery from fluoride and aluminium induced free radical liver toxicity in mice. *Fluoride* 2004;37(4):257-63.
- 10 Pillai KS, Mathai AT, Deshmukh PB. Effect of subacute dosage of fluoride on male mice. *Toxicol Lett* 1988;44:21-9.
- 11 Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. *Ind J Biochem Biophys* 1984;21(4):130-2.
- 12 Luck H. A spectrophotometric method for the estimation of catalase. In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. London: Academic Press; 1963. p. 886-7.
- 13 Pagila DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967;70:15869.
- 14 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- 15 Grünert RR, Philips PH. A modification of the nitroprusside method of analysis for glutathione. *Arch Biochem* 1951;30:217-25.
- 16 Roe JH, Küether CA. The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl- hydrazine derivatives of dehydroascorbic acid. *J Biol Chem* 1943;147:399-407.
- 17 Chinoy NJ, Mehta D. Effects of protein supplementation and deficiency on fluoride induced toxicity in reproductive organs of male mice. *Fluoride* 1999;32(4):204-14.
- 18 Chinoy NJ, Patel DK. Influence of fluoride on biological free radicals in ovary of mice and its reversal. *Environ Sci* 1998;6(3):171-84.
- 19 Chinoy NJ, Sharma AK. Amelioration of fluoride toxicity by vitamins E and D in reproductive functions of male mice. *Fluoride* 1998;31(4):203-16.
- 20 Chinoy NJ, Narayana MV, Dalal V, Rawat M, Patel D. Amelioration of fluoride toxicity in some accessory reproductive glands and spermatozoa of rat. *Fluoride* 1995;28(2):75-86.
- 21 Chinoy NJ, Shukla S, Walimbe AS, Bhattacharya S. Fluoride toxicity on rat testis and cauda epididymal tissue components and its reversal. *Fluoride* 1997;30(1):41-50.
- 22 Sun GF, Shen HY, Ding GY. Effects of extraneous GSH on toxicity and metabolism of fluoride [abstract]. *Proceedings of the XXth Conference of the International Society for Fluoride Research; 1994 Sept 5-9; Beijing, China*. Beijing: Organizing Committee of ISFR'94; 1994. p. 156-7.
- 23 Li J, Cao S. Recent studies on endemic fluorosis in China. *Fluoride* 1994;27(3):125-8.
- 24 Bian JC, Xian SM, Ye P, Liu YP, Liu Y, Ji QX. Determination and analysis of trace elements, anti-oxidation material and lipid metabolism in the patients with fluorosis [abstract]. *Proceedings of the XXth Conference of the International Society for Fluoride Research; 1994 Sept 5-9; Beijing, China*. Beijing: Organizing Committee of ISFR'94; 1994. p. 129-30.
- 25 Chinoy NJ, Patel DK. Ameliorative role of aminoacids on fluoride induced alterations in uterine carbohydrate metabolism in mice. *Fluoride* 1996;29(4):217-26.
- 26 Chinoy NJ, Mehta D. Beneficial effects of the amino acids glycine and glutamine on testis of mice treated with sodium fluoride. *Fluoride* 1999;32(3):162-70.
- 27 Reddy SG, Srikantia SG. Effect of dietary calcium, vitamin C and protein in development of experimental skeletal fluorosis. 1. Growth, serum chemistry and changes in composition and radiological appearance of bones. *Metabolism* 1971;20(7):642-9.
- 28 Chinoy NJ, Mehta D, Jhala DD. Effects of different protein diets on fluoride induced oxidative stress in mice testis. *Fluoride* 2005;38(4):269-75.