

## GLUTATHIONE METABOLISM IN RATS EXPOSED TO HIGH-FLUORIDE WATER AND EFFECT OF SPIRULINA TREATMENT

Toshi Kaushik,<sup>a</sup> Radhey Shyam,<sup>b</sup> Praveen Vats,<sup>b</sup> Shoba Suri,<sup>b</sup>  
MML Kumria,<sup>b</sup> PC Sharma,<sup>a</sup> Som Nath Singh<sup>b</sup>

Meerut and Delhi, India

**SUMMARY:** Effects of high fluoride intake through water on glutathione and related enzymatic activities in blood and liver of albino rats were studied. Twenty four rats were divided into three groups of 8 each. Group I was given normal municipal supply water (fluoride content 0.55 ppm), Groups II and III were exposed to 12 ppm fluoride in water for 15 days. Group III was treated orally with Spirulina<sup>®</sup> (200 mg/kg bwt), a functional food rich in protein, vitamins and minerals, for study of protective effects. After 15 days of exposure reduced and oxidised glutathione (GSH and GSSG), lipid peroxidation and enzymes, i.e. glutathione reductase (GR), glutathione peroxidase (GPx), glutathione S-transferase (GST), and  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT) activities were measured in blood/erythrocytes and liver. There was a significant rise in blood GSSG level and a decrease in GSH/GSSG ratio, with increased lipid peroxidation in fluoride-exposed animals. A marked decrease in GR and GST activities and an increase in  $\gamma$ -glutamyl transpeptidase activity were also noted in blood of fluoride exposed animals. In the liver no significant changes in these variables were observed. Results indicate oxidative stress during fluoride exposure. Spirulina treatment was beneficial to some extent as a rich source of the antioxidant vitamin  $\beta$ -carotene.

Keywords: Albino rats, Enzyme activities, Fluorosis, Glutathione metabolism, Lipid peroxidation, Spirulina.

### INTRODUCTION

Fluorosis, a disease or state of chronic poisoning from long-term exposure to excessive quantities of inorganic compounds of fluorine, is a serious health problem in countries where the fluoride content of water is more than 1 ppm. An estimated 62 million people in India in 17 states are afflicted with dental, skeletal, and/or nonskeletal fluorosis.<sup>1</sup> Fluorosis was first described in India by Shortt *et al*<sup>2</sup> in 1937 in both humans and farm animals. In Andhra Pradesh, knock-knee syndrome, commonly known as genu valgum is a serious problem that emerged in the mid 1970s, and large numbers of adolescents and young adults are affected (prevalence ranging from 0.2 to 17%).<sup>3</sup>

Fluorosis can be prevented through certain interventions if the disease is diagnosed at an early stage, and out of these interventions, nutritional measures have much importance. Consumption of a diet adequate in protein, calcium, vitamin C, vitamin E, and other antioxidants can minimise the adverse effects of fluoride.<sup>4</sup> Here we have studied the effect of high-fluoride con-

<sup>a</sup>Dept. of Biotechnology, Chaudhary Charan Singh University, Meerut-250 004, India.

<sup>b</sup>For correspondence: Dr Som Nath Singh, Nutrition Division, Defence Institute of Physiology and Allied Sciences, Timarpur, Delhi-110 054, India. Email: dipnut@rediffmail.com

sumption on glutathione levels and related enzymes. Glutathione is a multi-functional tripeptide that is a powerful antioxidant and a key molecule in xenobiotic drug metabolism.<sup>5-7</sup> The effect of Spirulina®, a product derived from the genus *Spirulina sp* and used as food supplement owing to its high protein, vitamin, and mineral content,<sup>8,9</sup> was also evaluated for protective effects against fluoride toxicity.

#### MATERIALS AND METHODS

Male albino rats (Sprague-Dawley strain), each weighing ~ 200 g, were maintained in the animal house of the Defence Institute of Physiology and Allied Sciences at  $22 \pm 1^\circ\text{C}$  with 12-hr light/dark cycle and fed *ad libitum* on standard pellet diets supplied by Lipton India Ltd. Twenty-four rats were divided into 3 groups of 8 animals each. Group I (control) was given normal municipal supply water (fluoride content 0.55 ppm). Groups II and III were exposed to high fluoride for 15 days by supplementing the municipal supply water with NaF to give a fluoride concentration of 12 ppm. Group III was treated orally with 200 mg of Spirulina®/kg bwt for 15 days along with high-fluoride water.

After 15 days animals of all 3 groups were sacrificed with an overdose of ether anesthesia after overnight fasting. Blood was drawn from the heart. The liver was removed and kept on ice till further processing. An aliquot of blood (50  $\mu\text{L}$ ) was taken directly into a tube containing 0.45 mL of 10% (w/v) metaphosphoric acid (MPA) for estimation of reduced and oxidised glutathione (GSH and GSSG). Similarly, small portions of liver (~ 250 mg) were homogenised in 10% MPA for fluorometric estimation of GSH and GSSG.<sup>10</sup> Plasma was separated by centrifugation (1000 rpm  $\times$  10 minutes) at  $4^\circ\text{C}$ , and erythrocytes were washed three times with ice-cold 150 mM KCl and lysed in 50 mM KCl for enzymatic studies.

Liver homogenates (10% w/v) were prepared in 150 mM KCl. Two-mL aliquots of crude homogenate were taken for estimation of lipid peroxidation as 2-thiobarbituric acid reactive substances (TBARS).<sup>11</sup> Remaining liver homogenates were centrifuged at  $3000 \text{ g} \times 15$  minutes at  $4^\circ\text{C}$ , and supernatants were stored at  $-20^\circ\text{C}$  and used later for estimation of enzymes. Enzymatic activities of glutathione peroxidase (EC 1.11.1.9), glutathione reductase (EC 1.6.4.2), glutathione S-transferase (EC 2.5.1.18), and  $\gamma$ -glutamyl transpeptidase (EC 2.3.2.2) were estimated in RBC lysates and liver homogenates using standard methods.<sup>12-15</sup> Protein content of samples was measured colorimetrically by the method of Lowry *et al.*<sup>16</sup>

Data were expressed as Mean  $\pm$  SEM. Statistical analysis was done using unpaired 't' tests. Comparisons were made between fluoride-exposed and unexposed animals and between fluoride-exposed *vs* spirulina-treated fluoride-exposed animals. A p value of  $<0.05$  was considered significant.

### RESULTS AND DISCUSSION

Plasma protein contents of the control, fluoride-exposed, and the fluoride-exposed spirulina-treated rats are given in Table 1. There was not much change in total plasma protein, although the spirulina-treated group showed a slight increase in protein content. Spirulina is 70% protein, but since the dose used in the present study was only 200 mg/kg bwt or about 40 mg/rat/day, this amount is unlikely to be the cause of increased plasma protein content. Similar studies on human subjects by Uslu<sup>17</sup> and by Srikantia and Siddiqui<sup>18</sup> also indicated no change in protein content, haematocrit, and haemoglobin during high-fluoride intake. Altered plasma protein content has been found in two fluoride toxicity studies on animals.<sup>19-20</sup>

**Table 1.** Changes in plasma protein levels

Group	g/dL
Control	7.312 ± 0.19
Fluoride-exposed	7.190 ± 0.40
Fluoride-exposed & spirulina-treated	7.710 ± 0.18

Values are Mean ± SEM (n=8).

**Table 2.** Changes in reduced glutathione (GSH), oxidised glutathione (GSSG) and GSH/GSSG ratio. Values are expressed in µmol/mL for blood and µmol/g for liver

Group	GSH		GSSG		GSH/GSSG Ratio	
	Blood	Liver	Blood	Liver	Blood	Liver
Control	1.098 ± 0.321	4.711 ± 0.309	0.065 ± 0.003	0.447 ± 0.006	16.89	10.54
Fluoride-exposed	1.002 ± 0.053	4.196 ± 0.104	0.114 ± 0.007*	0.454 ± 0.021	8.79*	9.24
Fluoride-exposed & spirulina-treated	0.942 ± 0.046	5.003 ± 0.288†	0.089 ± 0.004*†	0.495 ± 0.027	10.58*	10.11

\*p<0.001 in comparison with control. †p<0.05, ‡p<0.01 in comparison with fluoride-exposed. Values are Mean ± SEM (n=8).

**Table 3.** Changes in lipid peroxidation levels in liver and blood

Group	Liver (nmol/g)	Blood (nmol/mL)
Control	38.052 ± 4.468	0.895 ± 0.057
Fluoride-exposed	40.487 ± 4.254	2.254 ± 0.101*
Fluoride-exposed & spirulina-treated	33.480 ± 3.553	2.455 ± 0.035*

\*p<0.001 in comparison with control. No significant change in fluoride-exposed vs spirulina-treated. Values are Mean ± SEM (n=8).

**Table 4.** Changes in enzyme activities of glutathione metabolism in liver and erythrocytes. Values are expressed as nmol/min/mg protein

Group	GR		GPX		GST		γ-GT Liver
	Liver	RBC	Liver	RBC	Liver	RBC	
Control	17.158 ± 1.108	4.982 ± 0.775	238.946 ± 23.303	69.636 ± 15.004	164 ± 11	64 ± 4	0.271 ± 0.037
Fluoride- exposed	19.254 ± 1.761	1.308 <sup>†</sup> ± 0.300	248.946 ± 34.158	55.376 ± 9.285	166 <sup>†</sup> ± 15	10 ± 2	0.481 <sup>†</sup> ± 0.020
F-exposed & spirulina-treated	16.377 ± 0.912	2.09 <sup>*</sup> ± 0.315	343.04 ± 32.307	55.262 ± 9.272	144 ± 21	14 <sup>†</sup> ± 5	0.588 <sup>*</sup> ± 0.078

\*p<0.01, †p<0.001 in comparison with control. No significant change in fluoride-exposed vs spirulina-treated. Values are Mean ± SEM (n=8).

Reduced glutathione levels in liver of the three groups did not differ significantly. However, a decrease in GSH of fluoride-exposed and an increase in spirulina-treated animals was observed (Table 2). Oxidised glutathione (GSSG) of blood was increased significantly in fluoride-exposed animals in comparison with the control group. Spirulina-treated animals showed a smaller increase in GSSG in comparison with untreated animals, thereby indicating a protective effect. The GSH/GSSG ratio in blood was also decreased significantly in fluoride-exposed animals, but in the spirulina-treated group the decrease was less in comparison with the control animals. In the case of the liver no such changes occurred. There glutathione is present mainly in reduced form (90-100%), in such large amounts that it maintains the reducing state of the living cell milieu.<sup>5</sup>

Whenever the GSH/GSSG ratio decreases, there is an adverse effect on several key enzymes of glycolysis.<sup>21</sup> Increased amounts of GSSG are transported out of cells to maintain the normal ratio.<sup>22,23</sup> But when accumulated inside the cell, GSSG creates oxidative stress, and various cellular components become vulnerable to damage by reactive oxygen species, mainly membrane lipids, proteins, and DNA. Lipid peroxidation was greater in fluoride-exposed animals, and a significant increase in TBARS levels (>2.5 times) in blood was noted. Spirulina treatment decreased the level of TBARS in the liver, but it was unable to provide protection in the blood (Table 3).

The GSH/GSSG ratio is maintained by enzymatic activities of GR and GPx. GR converts GSSG to GSH in the presence of NADPH, while GPx acts as an antioxidant. GSH also participates in the transport of aminoacids via the γ-glutamyl cycle and protein synthesis, wherein the first enzyme involved is γ-glutamyl transpeptidase. Glutathione S-transferase binds electro-

philic moieties and various toxicants/drugs with glutathione and offers a powerful mechanism for detoxification.<sup>24</sup> Activities of these enzymes were estimated to determine the effect of fluoride toxicity and are given in Table 4. Glutathione reductase activity was increased in liver and was decreased significantly in blood of fluoride-exposed animals. The decrease in GR activity in fluoride-exposed animals may be responsible for the higher levels of GSSG.

Glutathione peroxidase activity in the liver of fluoride-exposed spirulina-treated animals was increased by 37.7% in comparison with fluoride-exposed untreated animals. In the blood no significant change in GPx activities of the three groups was noted, but glutathione S-transferase activity was decreased significantly in fluoride-exposed animals. The decrease in GST activity was 84.4% and 78.1%, respectively, in fluoride-exposed untreated and spirulina-treated groups in comparison with the control group. In the liver, however, GST activity showed little change. This indicates that the liver is protected from oxidative damage to a great extent when the oxygen supply and energy production are optimum.<sup>25-27</sup> Decreased GST activity in blood following fluoride exposure is indicative of impaired drug metabolism and should be further investigated.

$\gamma$ -Glutamyl transpeptidase activity was increased significantly in the liver of fluoride-exposed and spirulina-treated groups when compared with the control group. Excessive formation of glutamine in skeletal muscle is reported in fluoride toxicity, indicating increased protein turnover. In the present study increased  $\gamma$ -GT activity supports increased protein turnover, although we could not detect much change in plasma protein levels.

Increased levels of glutathione (GSH) as protective phenomenon in response to high-fluoride intake have been reported by Sridharan *et al.*<sup>28</sup> In that study, however, oxidised glutathione levels and enzyme activities were not reported. Thus there is a need for study of this metabolism in human subjects in fluoride-endemic areas. Here we found that spirulina treatment of fluoride-exposed rats for 15 days indicated some beneficial effects, but long-term feeding may be required to achieve optimum benefit.

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