Assessment of health risks associated with fluoride-contaminated groundwater in Birbhum district of West Bengal, India

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Abstract

Fluoride contamination in drinking water is a public health problem in many areas around the world. Fluoride is known to cause diseases like dental fluorosis, Alzheimer's disease, dementia, skeletal fluorosis, abnormal thyroid function and other hormonal disturbances. In addition to fluoride-affected Indian states like Andhra Pradesh and Punjab, the specified regions of Birbhum and Purulia districts of West Bengal have already been declared to be fluoride-polluted by the Public Health Engineering Department. The aim of this study is to analyze the fluoride-induced oxidative stress damage parameters of the male inhabitants and to mitigate such impact by antioxidant vitamin supplementation. Seventy-five male dwellers residing at Noapara, Nasipur, Zunitpur, Bhabanandapur, Chakpara, and Atla of Birbhum district within the age group of 25-35 years were selected. They were randomly divided into three groups of 25 men each. Vitamin E (clinical dose) and a mixture of vitamin E, vitamin C, ß-carotene, and reduced glutathione, as well as peppermint lozenges (placebo) were respectively supplemented in the three groups for 21 days. The hemoglobin levels, body mass index, glucose-6-phosphate dehydrogenase level, and malondialdehyde status were assessed before and after supplementation. Standard methods were used to analyze the groundwater samples from these areas for their chloride, fluoride, and iron concentrations. The acute fluoride toxicity has been established in the groundwater samples. Results indicate that vitamin mixture and glutathione supplementation together may be treated as an important detoxification technique against detrimental effects of fluoride toxicity to male inhabitants, at least partially.

Keywords: Fluoride toxicity, groundwater, human health.

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1. Introduction

The Birbhum district, 4,545 km^2 with a population size of above 25 lakhs and with a density of population of 562/km^2, experiences a silent but inevitable natural disaster in the form of fluoride pollution in groundwater [1]. Amongst general land use and cropping patterns, a very small portion of forest exists and a huge area is considered arable, unirrigated type (Figure 1). There is a gradual rise in urbanized settlement and minimum rural settlement. Very few water bodies exist in the district. From the climatic condition and rainfall data, the district may be considered as primarily an arid zone (Figure 2) [2]. Fluorine, a gaseous element, is a halogen with a high electronegativity and reactivity and does not occur in free form in nature. Fluoride combines directly with most elements and immediately with few to form fluorides. Fluorides are ubiquitous in nature and are present in rocks, soils, water, plant, foods, and even air. Birbhum district and some specific parts of this area, particularly Noapara, Nasipur, Zunitpur, Bhabanandapur, Chakpara, and Atla are found to be extremely polluted with fluoridated groundwater [2]. The cause of such pollution is erosion of rock and soil in particular. Some scientists also describe anthropogenic origins, although those are found to be based on hypothesis, not experimental proof. From the geophysical point of view, the cause of fluoride pollution has been reviewed by scientists many times, but assessment of physiological damage by such fluoridated

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water has been scantily performed. A study showed that fluoride toxicity may lead to dental fluorosis, and be associated with Alzheimer's disease and other types of dementia, formation of a crippling bone disease called skeletal fluorosis, disruption of thyroid gland activity, and reduction in melatonin level [3]. Sometimes fluoride is considered a carcinogen and fluoride intake increases probability of hip fracture. Moreover, fluoride increases infertility [4]. Fluorosis and tooth decay compels the US Food and Drug Administration not to approve fluoride as a toothpaste ingredient [5]. Out and out, fluoride is considered as a toxic hazard to humans. Its neurotoxin nature, osteocircoma-producing activity, and parental exposure unequivocally establish it as the "silent criminal for damaging human beings" [6]. Surveys at Nasipur, Noapara, Chakpara, Atla, Bhabanandapur, and Junitpur villages have shown that most people suffering from fluorosis are very poor, and as such they do not use fluoridated dental products [3]. Hence, fluoride toxicity in those people is not fallout of fluoride-related products [3]. The present study incorporates some of the stringent symptoms of dental fluorosis (Figure 3) and skeletal fluorosis (Figure 4) as found in the dwellers of this region. Qualified dentists and physicians in their valued survey report, indicate the following symptoms of both types of fluorosis experienced by the dwellers of different age groups at varying degrees.

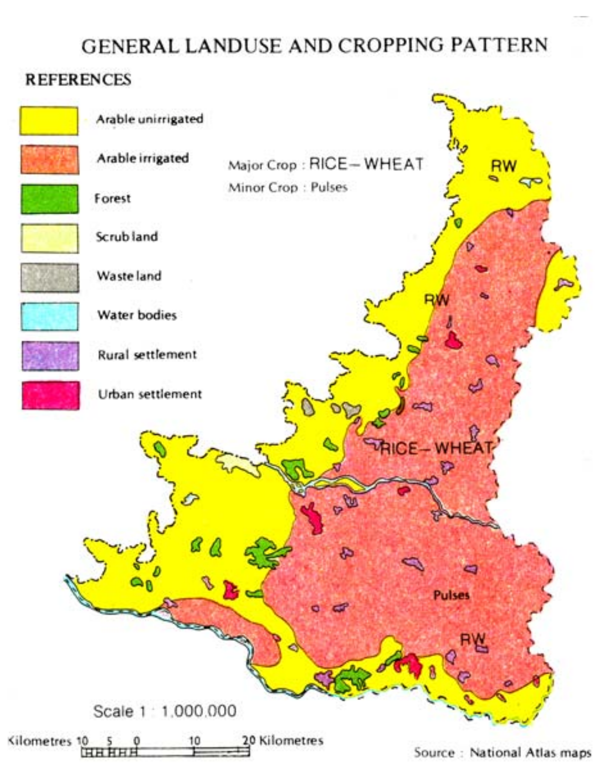

Figure 1. General land use and cropping pattern of Birbhum district showing the sites of experimental work.

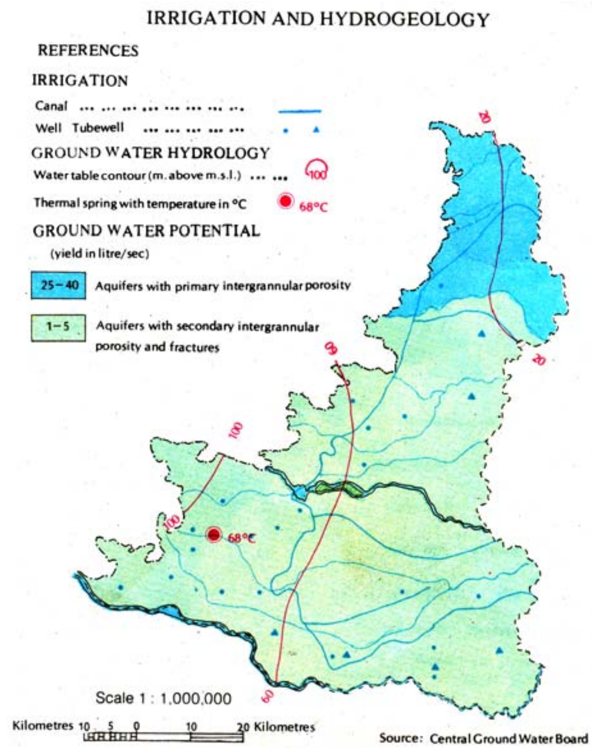

Figure 2. The irrigation and hydrogeology of Birbhum district and groundwater database.

Figure 3. Specific view of dental fluorosis.

Symptoms of dental fluorosis include lack of lustre, white patches, browning of teeth, pitting and swelling, pain and pus, and loss of teeth. Symptoms of skeletal fluorosis include tingling and numbing of extremities, joint pain, back pain, knee pain, shoulder pain, neck pain, pain in limbs, stiff vertebral column, inability to walk properly, kyphosis, difficulty sitting in a squatting position, inability to cross legs, fold hands, and get up from sitting posture.

Figure 4. Skeletal fluorosis developed in Nasipur village.

In the area under survey, almost 90% of the adults have been identified to suffer from skeletal fluorosis and sometimes spondylosis (degeneration of inter-vertebral discs), osteoporosis (bones become abnormally dense, splinter and fracture), and arthritis [7]. In early stages of such toxicity, the patients show symptoms of nausea, vomiting, abdominal cramps, muscular-fibrillation, and numbness of mouth [7]. There are cases of allergic reactions in some individuals with variations in susceptibility from patient to patient. The picture in Nasipur village is tragic. Many people become crippled and knock-kneed, with crumpled fingers and mottled teeth. People at advanced stages of fluorosis in this region cannot pass urine or clean themselves [2]. The cause of high fluoride in the groundwater of Birbhum district and its resultant toxicity in humans can be considered a lithology-controlled medico geological problem of groundwater toxicity [8]. The problem, therefore, needs to be addressed and resolved from this point of view. This study specifically aims to compare groundwater quality of selected areas of Birbhum district and that of Kolkata (as a control) indicating the fluoride, iron, and chloride status in particular. Further, the study attempts to highlight and compare the damaging effects of excess fluoride intake since birth on general health, to specify the comparative damage profile of excess fluoride on hemoglobin status, and to confirm the level of mitigation of oxidative stress thus incurred, by biological supplementation. Serum iron status is monitored as it is directly correlated to hemoglobin level. Chloride, unlike fluoride, is the most abundant anion present in body fluids and as it belongs to the halide family, it needs to be assayed to find out whether excess fluoride intake alters its status or not.

2. Materials and Methods

2.1 Collection of groundwater samples

Samples were collected from the following locations: Noapara, Nasipur, Zunitpur, Bhabanandapur, Chakpara, and Atla villages in Birbhum district.

After rejection of continuous flowing water from the tube-well tap, more than 0.5 liter of water was taken aseptically in a sterile glass container and marked accordingly. Sampling was performed in villages of Birbhum district concerned and also in the College Street regions of Kolkata using only tube-wells (Table 1). Water sampling in Kolkata was performed to obtain control data.

2.2 Analysis of water samples

All water samples were chemically analyzed according to the methods standardized by "American Public Health Association" guidelines. The analysis for physico-chemical parameters and fluoride in ground and surface water samples was carried out according to the procedure outlined in standard methods [9]. Fluoride (F^-) was determined by sodium-2-(parasulphophenylazo)-1,8-dihydroxy-3,6-napthalene disulphonate reagent method using colorimetry.

2.3. Human sampling

Seventy-five male inhabitants from Birbhum district were randomly chosen for the double-blind study. Since several studies already showed that people residing in and drinking the groundwater of Kolkata are not susceptible to any forms of fluorosis, human sampling in Kolkata was ignored. However, the study was performed following the ethical guidelines for biomedical research on human participants as directed by the Indian Council of Medical Research and was duly approved by the departmental "Research Ethics Board".

Study participants were asked to refrain from taking extra vitamin supplementation three months before and during the study. None of these subjects had any history of chronic cardiac or respiratory diseases. The entire study was performed with the permission of the government authority (District Magistrate) and all the subjects were told the objectives and probable impact of the work. All the ethical formalities were performed in writing and written consent of all the subjects was also obtained. They were informed about the objectives of the study and about the probable outcomes of the vitamin and glutathione (GSH) supplementation in a lucid way, and they unanimously agreed to volunteer for the study. All the workers were supplied with the required amount of vitamin capsules during the entire experimental tenure. A double-blind study was carried out whereby the subjects were divided into three groups. Two groups each of 25 subjects were randomly assigned for experimental groups A and B, and were given 400 mg of pure vitamin E (Evion 400 tablet) only and a mixture of vitamin E, vitamin C, β-carotene (E-carotin tablet), and 50 mg reduced GSH (incyto tablet), respectively, for a period of 21 days.

Table 1. Location of sampling points.

Sample	Sources	Collection date	Location	Block
1	Hand pump no. 1	10.03.08	Noapara	Rampurhat-1
2	Hand pump no. 2	10.03.08	Chakpara	Rampurhat-1
3	Hand pump	10.03.08	Atla	Rampurhat-1
4	Shallow tube well no. 1	10.03.08	Noapara	Rampurhat-1
5	Shallow tube well no. 2	10.03.08	Noapara	Rampurhat-1
6	Hand pump no. 2	10.03.08	Noapara	Rampurhat-1
7	Pond	10.03.08	Noapara	Rampurhat-1
8	Hand pump no. 3	10.03.08	Noapara	Rampurhat-1
9	Hand pump no. 1	10.03.08	Junitpur	Rampurhat-1
10	Hand pump no. 2	10.03.08	Junitpur	Rampurhat-1
11	Shallow tube well no. 1	10.03.08	Junitpur	Rampurhat-1
12	Pond	10.03.08	Junitpur	Rampurhat-1
13	Hand pump no. 3	10.03.08	Junitpur	Rampurhat-1
14	Shallow tube well no. 2	10.03.08	Junitpur	Rampurhat-1
15	Tap water (PHE)	15.03.08	Bhabanandapur	Nalhati-1
16	Well	15.03.08	Bhabanandapur	Nalhati-1
17	Well	15.03.08	Bhabanandapur	Nalhati-1
18	Well	15.03.08	Bhabanandapur	Nalhati-1
19	Hand pump no. 1	15.03.08	Bhabanandapur	Nalhati-1
20	Well	15.03.08	Bhabanandapur	Nalhati-1

Experimental group C consisting of 25 subjects of the placebo group was also studied. Peppermint lozenges were given to the placebo group daily for 21 days.

2.4 Assessment of biochemical parameters

Blood was taken in fasting condition for the determination of hemoglobin (gram percent: g%) by the cyanomethaemoglobin method [10]. The level of serum lipid peroxidation was estimated in terms of serum malondialdehyde (MDA) by the method of Yagi [11], using thiobarbituric acid. Assessment of glucose-6-phosphate dehydrogenase (G-6-PD) enzyme (quantitative) was also carried out with the blood drawn before and after supplementation by UK-kinetic method [12]. This study attempts to quantify the "fluoride induced oxidative stress" production in the body, by virtue of assessing "serum MDA level", which is an index of stress-induced lipid peroxidation [13]. Statistical analysis was done using a two-tail 't' test by the difference method. The Student's t-test for independent samples was used to test differences between different groups regarding serum MDA level, blood G-6-PD level, and endurance capacity. For these variables, within-group differences from baseline to week 3 were evaluated using the Student's *t*-test for matched-pair samples. When associations between antioxidant supplementation and the variables were evaluated, the baseline levels of all the study subjects were used.

Radiographs were obtained from different limb structures (Figures 5 and 6). The statistical analyses were performed on all the data generated in the present study using a statistical software, namely Statistical Package for the Social Sciences for Windows, version 11.0.1. A *p*-value of < 0.05 was considered statistically significant.

3. Results and Discussion

Geochemically, tropical environments are unique. This uniqueness stems from the fact that these terrains are continuously subjected to extreme rainfall and drought with resulting strong geochemical fractionation of elements. This characteristic geochemical partitioning results in either severe depletion of elements or accumulation to toxic levels. In both these situations, the effect on plant, animal, and human health is marked. Medical geochemistry involves the study of the relationships between the geochemistry of the environment in which we live and the health of the population living in this particular domain. Interestingly, the relationships between geochemistry and health are most marked in the tropical countries, which coincidentally are among the poorest in the world. In terms of geological age in Karbianglong and adjoining areas of Assam, rock assemblages range from Archean to Recent with a significant proportion of economically important limestone, coal, and clay deposits. The pink and purple granite in the district also contain a significant proportion of fluoride-containing minerals, such as apatite [14]. Due to its interaction, the fluoride is released into soil and groundwater [15]. The permissible limit of fluoride, according to the US Environmental Protection Agency, in drinking water is 1 ppm. Birbhum district and some specific parts of this area – particularly Noapara, Nasipur, Zunitpur, Bhabanandapur, Chakpara, and Atla are found to be extremely polluted with fluoridated groundwater. From the anthropometric point of view, body mass index (BMI) below 20 is considered as low compared to the standard value. All three groups studied have comparable BMI values as is apparent from the statistical treatment (Table 4, Figure 7).

Figure 5. Cervical spine lateral view – showing loss of lordosis in lumber spine. Posterior osteophyte seen in C4-C6 and C6-C7 disc spaces are diminished ensuring spondylosis possibly due to skeletal fluorosis.

Nasipur and Noapara regions of Birbhum district, West Bengal, seem to be suffering from geochemical fluoride contamination in regional groundwater (Tables 2 and 3; Figures 8-10) and the probable origin of such excess fluoride, geologists believe, is volcanic eruption of basaltic composition. The basaltic rocks are exposed to surface. They are highly weathered, indicating that they are from the adjoining Rajmahal volcanics.

Figure 6. Cervical spine lateral view – showing posterior osteophyte in C7 body. C7 to T1 disc space is diminished ensuring spondylosis due to skeletal fluorosis.

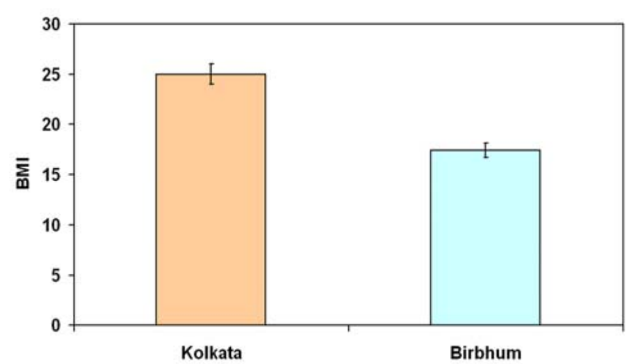

Figure 7. Comparative BMI study.

Table 2. Analysis of groundwater samples collected from affected areas of Birbhum district.

Sample no.	Source	Fe^{2+} (ppm)	Cl^{-} (ppm)	F^{-} (ppm)
1	Hand pump no. 1	0.16	7	**2.67**
2	Hand pump no. 2	0.33	9	**6.88**
3	Hand pump no. 3	0.79	7	**4.67**
4	Shallow tube well no. 1	1.5	5	**1.13**
5	Shallow tube well no. 2	0.06	106	**1.07**
6	Hand pump no. 4	2.18	7	**2.45**
7	Shallow tube well no. 3	0.25	3	0.18
8	Hand pump no. 5	3.10	5	**2.00**
9	Hand pump no. 6	0.37	120	0.35
10	Hand pump no. 7	0.49	9	0.90
11	Shallow tube well no. 4	0.14	52	**2.25**
12	Shallow tube well no. 5	0.72	31	0.42
13	Hand pump no. 8	3.10	5	**2.00**
14	Shallow tube well no. 6	0.13	52	**2.25**
15	Well 1	0.41	120	0.35
16	Well 2	4.3	9	**1.11**
17	Well 3	1.80	7	**1.65**
18	Well 4	0.01	5	**1.13**
19	Hand pump no. 9	3.18	5	**1.00**
20	Well 5	0.78	9	**1.50**
Mean ± SD		1.19 ± 0.20	28.65 ± 0.22	1.798 ± 0.18

Table 3. Comparative analysis of groundwater samples collected from Kolkata and the affected areas of Birbhum district.

Chemical parameters	Groundwater of Kolkata	Groundwater of Birbhum district
Fe^{2+} (ppm)	1.9 ± 0.31	1.19 ± 0.20
Cl^{-} (ppm)	290.00 ± 16.43	28.65 ± 0.22
F^{-} (ppm)	0.2 ± 0.04	1.798 ± 0.18

The village Nasipur lies on the Rajmahal trap consisting of fractured and jointed rock. Fresh basalt exists at a depth of about 20 to 30 m and is found in a nearby quarry. It is fine grained, hard, and compact. Therefore, the physical characteristics of the subjects have no impact on the changes in the biochemical parameters observed due to supplementation, indicating the unbiased nature of the study. Hematologically, the subjects showed hemoglobin titer ranging from 11.6 to 12.2 g%, indicating that excess fluoride intake incidentally did not make the subjects anemic, at least in the age group studied. Further, the study ensures that hemoglobin status in all three groups does not show any significant change after 21 days of supplementation (Table 5). Geologically as well as anthropologically polluted groundwater itself is supposed to be an oxidative stress to humans, as a consequence of which it produces reactive oxygen species (ROS) in the body, igniting the antioxidant defence within itself [16, 17]. Earlier reports proved that any unnatural fluid intake may elevate the stress indices like plasma lipid peroxides and higher blood GSH oxidation [17].

Table 4. Physical parameters of subjects studied.

Sl. no.	Age (years)[a]	Sex	Height (cm)[a]	Weight (kg)[a]	BMI (kg/m^2)[a]
A	30 ± 3	M	162.5 ± 3.83	50.21 ± 2.16	18.93 ± 1.218
B	28 ± 2	M	165.46 ± 2.15	51.34 ± 1.19	18.37 ± 1.01
C	30 ± 2	M	162.45 ± 3.35	50.95 ± 2.91	18.94 ± 1.37

[a] Values are mean ± S.D.

Table 5. Hemoglobin concentration of males before and after antioxidant vitamin supplementation.

Experimental group	**Hemoglobin concentration (g%)**	
	Pre-supplementation[a]	Post-supplementation[a]
A	11.6 ± 0.6	11.6 ± 0.6
B	12.2 ± 0.4	12.2 ± 0.4
C	12.0 ± 0.3	12.0 ± 0.3

[a] Values are mean ± S.D.
No statistical significance was found between pre- and post-supplemented subjects ($p > 0.05$).

Table 6. Serum lipid peroxidation levels of males before and after antioxidant vitamin supplementation.

Experimental group	**Serum lipid peroxidation level ($k\times10^{-1}$ nmol/ml of serum)**	
	Pre-supplementation	Post-supplementation
A	32.91 ±2.12	31.45 ± 2.30
B	33.43 ±1.50	29.52* ± 1.76
C	33.13 ±1.25	32.89 ± 1.34

[a] Values are mean ± SD.
* Statistically significant difference between pre- and post-supplemented subjects ($p < 0.001$).

Table 7. G-6-PD of males before and after antioxidant vitamin supplementation.

Experimental group	G-6-PD (U/g Hb)	
	Pre-supplementation	Post-supplementation
A	9.31 ± 0.74	10.86* ± 0.82
B	9.34 ± 0.78	11.43** ± 0.54
C	9.30 ± 0.60	9.21 ± 0.54

[a] Values are mean ± S.D.
Statistically significant difference between pre- and post-supplemented subjects (*$p < 0.05$; **$p < 0.01$).

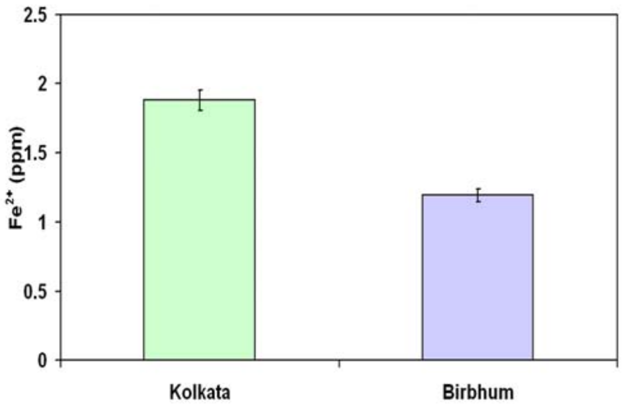

Figure 8. Comparative bar diagram of Fe^{2+} status with groundwater of Birbhum and Kolkata.

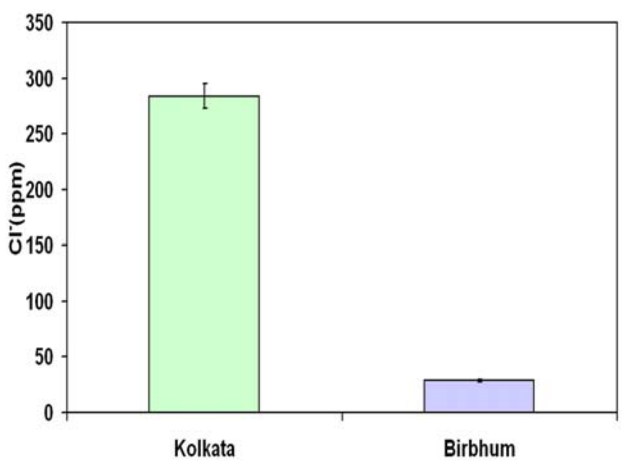

Figure 9. Comparative bar diagram of Cl^- status with groundwater of Birbhum and Kolkata.

ROS are known to have a wide variety of patho-physiological characteristics [18]. ROS is responsible for lipid, protein, and deoxyribonucleic acid (DNA) damage in a major way. Lipid peroxidation produces conjugated dienes, lipid hydroperoxides, and MDA. All these lead to diseases. However, MDA is often assessed clinically as an indicator parameter or marker of oxidative stress. Moreover, ROS may also contribute to fatigue in oxidative skeletal muscle [19]. Skeletal fluorosis and dental fluorosis are found to be a common ailment among the villagers there. Recently, a number of studies have been directed towards an exercise-induced oxidative stress management program. Antioxidant supplementation studies have revealed a beneficial trend [16, 17].

Antioxidants like vitamin E, vitamin C, and GSH are known to act synergistically [17, 20] in the form of antioxidant chain reaction [17]. Lipophilic vitamin E is a major lipid peroxidation chain-breaking antioxidant. The water-soluble antioxidants like ascorbate and GSH may be involved in regenerating α-tocopherol from its radical by-products like tocopherol 0, *etc.* [20]. In plasma, vitamin C is a potent antioxidant, the first to be depleted on exposure to peroxyl radicals and other types of oxidative stress [21].

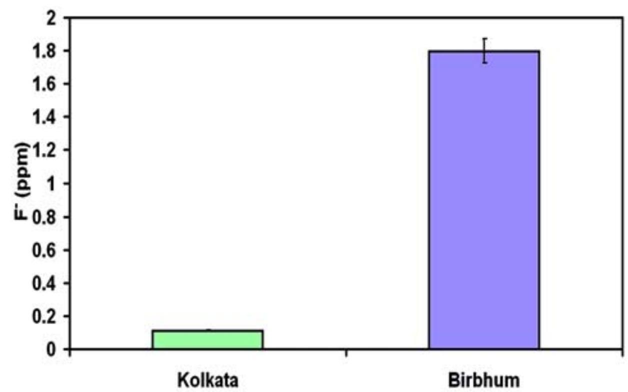

Figure 10. Comparative bar diagram of F^- status with groundwater of Birbhum and Kolkata.

The study is unique in supplementing β-carotene, along with vitamin E and vitamin C, where β-carotene actively scavenges and deactivates free radicals both *in vitro* and *in vivo*. Additionally, it acts as a singlet oxygen quencher. Antioxidant vitamin mixture supplementation reduced the basal status of "serum lipid peroxidation" ($p < 0.001$). On the other hand, it was observed that vitamin E supplementation alone failed to reduce the MDA status (Table 6; Figure 11). In the placebo group C, however, no such post-supplemental changes were observed.

G-6-PD is the rate-limiting enzyme in the first step of the pentose phosphate pathway, which is essential for the production of nicotinamide adenine dinucleotide phosphate (NADPH) in the red blood cells for the recycling of endogenous antioxidant GSH [22]. Some of those NADPH importantly neutralize hydrogen peroxide, GSH, and other organic peroxides produced in the oxidative stress [23]. This information made the G-6-PD level an even more important marker for fluoride toxicity-related oxidative stress management system.

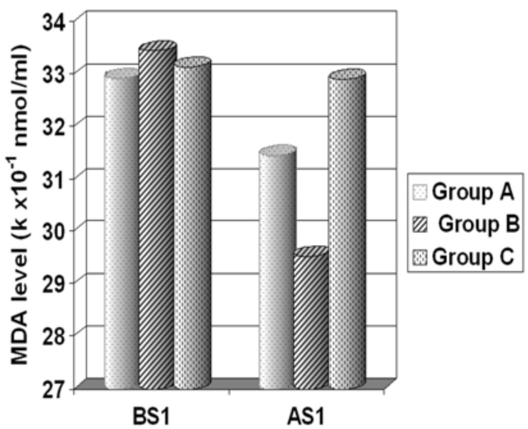

Figure 11. Serum lipid peroxidation levels (k × 10^{-1} nmol/ml of serum) of males before and after antioxidant vitamin supplementation**.** Key: BS1 = before supplementation; AS1 = after supplementation pre-exercise values.

G-6-PD level showed a more significant ($p < 0.001$) increase after antioxidant vitamin mixture along with GSH supplementation as compared to supplementation of vitamin E alone ($p < 0.05$). G-6-PD level increased from 9.31± 0.74 to 10.86 ± 0.82 in group A while the value increased from 9.34 ± 0.78 to 11.43 ± 0.54 in experimental group B. In placebo group C, however, there was no change (Table 7; Figure 12). Since all the values of group A, group B, and group C were found to be comparable and were not suffering from group-specific bias after statistical treatment, as depicted from Tables 1-6, it may be inferred that the differences observed after supplementation are exclusively due to the vitamin and GSH management.

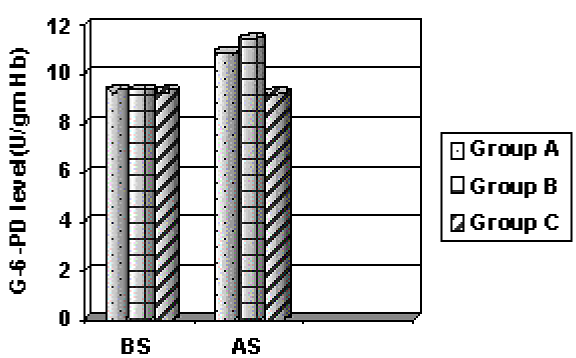

Figure 12. G-6-PD of males before and after antioxidant vitamin supplementation. Key: BS = before supplementation values; AS = after supplementation values.

4. Conclusion

The study confirms the dreaded impact of fluoride toxicity, in particular, on human health. The groundwater quality of Nasipur and Noapara of Birbhum district was found to be highly polluted from excess fluoridation and devoid of potable characteristics of water. Therefore, the residents are compelled to swallow this anthropogenically and geologically polluted water since their birth. From the physiological and clinical point of view, almost 95% of the subjects were found to suffer from dental and skeletal fluorosis. Some of the photographs, taken by us, ensure the fact. Immediate arrangement for defluoridation and potable groundwater availability should be taken from the governmental point of view at a larger scale. Antioxidant vitamins along with GSH help to act as an effective protocol in "oxidative stress management," at least partially, on fluoride-exposed individuals, specifically by increasing G-6-PD level. It may be hypothesized that the antioxidant activity within the erythrocytes may be related to changing level of oxidative stress within the red blood cells, as evident from the levels of G-6-PD itself. Malnutrition and vitamin supplementation may be the regulating feature for such activity. Further research is necessary in this exciting area. Antioxidant vitamins, especially an antioxidant vitamin mixture along with reduced GSH, effectively reduces the stress level of the fluoride-affected individuals as evidenced by a reduction in the basal status of lipid peroxidation (serum MDA level), thus indicating an alternative approach in bioremediation of fluoride-stressed personnel. However, the clinical setting of these beneficial findings has not yet been established.

5. References

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