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Amelioration of fluoride toxicity using amla (*Emblica officinalis*)

Sumeet Ranjan and Shahla Yasmin*

Department of Zoology, Patna Women's College, Patna 800 001, India

A study was conducted on the residents of Bhupnagar, a fluoride endemic village in Gaya district, Bihar, to assess the ameliorating effect of amla (*Emblica officinalis*) on fluoride-induced toxicity. Fifty-three subjects of different age groups were included in the study. Among these, 27 subjects (test group) were given amla powder as dietary supplement for 9 months and the rest 26 subjects were kept as control (i.e. without dietary supplement). The mean urinary fluoride level in the test group was found to decline progressively during the period of supplementation. The study revealed that consumption of amla powder may play an important role in mitigating fluoride-induced toxicity.

Keywords: Amla powder, ameliorating effect, dietary supplement, fluoride toxicity.

FLUORIDE, ingested through water and food accumulates in the human body and causes fluorosis. Fluoride may enter the body through sources other than drinking water, viz. (i) food and food products, spiced with black rock salt with 157 mg/l fluoride, (ii) use of fluoridated dental products, (iii) industrial emission of fluoride dust and fumes[1], and (iv) chewing supari and tobacco which have high fluoride content[2]. The maximum acceptable limit of fluoride in drinking water according to BIS[3] is 1.0 mg/l.

Bhupnagar village in the Amas Block of Gaya district, Bihar, is a fluoride endemic village, with mean F levels = 2.857 ± 0.11 mg/l. It was found that in this area, 47% of the entire fluoride intake for an adult was through drinking water followed by cereals and pulses (28%), and vegetables (25%)[4]. The residents of this village were found to suffer from dental, skeletal and non-skeletal fluorosis[5]. Anomalies were also found in the thyroid function[6] and haematological parameters[7] of the residents of Bhupnagar village.

As there are no medicines and/or surgical interventions to recover from fluorosis, the only alternatives are: (i) diet editing, where all sources of fluoride entry are withdrawn, and (ii) diet counselling for improving the health through consumption of essential nutrients and vitamins, antioxidants and micro nutrients. Vitamin C is one of the essential nutrients that would ameliorate fluorosis. Further, vitamin C enhances the absorption of nutrients by the gastrointestinal track[8].

Epidemiological studies in India[9] and Japan[10] suggest that the incidence and severity of fluorosis are related to the economic and nutritional status of the community. It

*For correspondence. (e-mail: shahla_apex@yahoo.co.in)

has also been observed that the bone deformities due to fluoride toxicity are more commonly found in the poorer and undernourished population[11,12]. Poor nutrition, including deficiencies in protein, vitamin C and calcium aggravates fluoride toxicity[13–15]. Therefore, it has been suggested that fluoride toxicity or fluorosis can be prevented and controlled by consuming safe water and nutritional supplements[8,16,17]. It has also been reported that the combined supplementation of calcium with vitamin C can help in the reversal of fluoride-induced alteration in the structure and metabolism of soft tissues[18,19]. Another study reported that individuals consuming citrus fruits and having good nutritional status suffer least from fluorosis[20]. A survey showed that the occurrence of fluorosis was higher in the people taking poor diet (including only cereals), compared to those including milk, eggs, and citrus fruits in their diet, although both groups were exposed to roughly the same fluoride level in groundwater[21].

A defluoridation unit with reverse osmosis technology was installed in the Bhupnagar village in 2012, to provide fluoride safe water for drinking and cooking purposes. Keeping in view the low socio-economic status of the villagers, a study was planned where a group of subjects was convinced to include amla (*Emblica officinalis*) powder in their daily diet. Pharmacological studies have shown that amla possesses antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic, cardioprotective, gastroprotective, antianemia, antihypercholesterolemia, wound healing, antidiarrhoeal, antiatherosclerotic, hepatoprotective, nephroprotective and neuroprotective properties[22]. This communication presents results of the possible ameliorative action of amla in managing fluorosis.

Fifty-three residents from the village volunteered to participate in the study. They were divided into two groups, one with 27 subjects as test group and another with 26 subjects as the control group. Individuals of both the test and control groups were age–sex matched. The 27 test subjects were instructed to consume only defluoridated water from the filtering unit. The subjects of the control group consumed the defluoridated water, but took no dietary supplements.

The subjects of the test group consumed dry amla powder (one teaspoon ~5 mg) twice a day with lukewarm water. Dry powder (amla churan manufactured by Divya Pharmacy) was used instead of fresh fruit, because the latter is available only seasonally for a short period of time. A 100 g fruit pulp of amla has been found to contain 600 mg of vitamin C[23]. On drying, the vitamin C content is not depleted[24]. Therefore, on an average each test fluorotic subject consumed roughly 60 mg of vitamin C in the form of amla. The daily intake of ascorbic acid has been fixed at 40 mg by ICMR in 2010. However, according to Carr and Frei[25], the recommended dietary allowance (RDA) for vitamin C for adult non-smoking men and women is 60 mg/day, because ~85% of the vitamin C is absorbed at usual intake. As urine is the main excretion route for the ingested fluoride, analysis of fluoride concentration in urine is a useful indicator of fluoride load in the study subjects[26]. Although 24 h urine is considered to be reliable[27], it was not practical to collect such samples from the remote village. Therefore, a spot sample of urine was collected for testing.

The study was carried out from March to November 2014. The urine samples were analysed every month on the same date. The spot urine samples were collected between 10.00 and 11.00 am in plastic bottles and fluoride level was measured by the ion selective electrode. Data were analysed with repeated measures ANOVA. Post test for linear trend was also performed. GraphPad Prism 5 was used for data analysis.

There are only two sources of drinking water in Bhupnagar – hand pump and defluoridation unit installed by the Bihar Government. Unfortunately, there is no alternative water supply. Rainwater harvesting is yet to begin in this area. Fluoride level was found to be 2.7 mg/l in one of the ponds in the village. The mean fluoride level in the groundwater of Bhupnagar village was 3.18 ± 0.29 mg/l (range 1.7–7.2 mg/l). The fluoride level of water from the defluoridation unit was 0.82 ± 0.006 mg/l. Gastrointestinal complaints were reported by all the test and control subjects prior to dietary intervention. But, in 16 out of 27 test subjects, there was relief from such problems and their appetite increased at the end 9 months (Table 1).

Fluoride in urine should normally be below 0.1 mg/l (ref. 8). Before the dietary intervention, in all the test subjects the urinary fluoride level was above this normal limit (Table 2). There was significant reduction in the mean fluoride level in the urine of subjects who consumed amla powder as dietary supplement ($F = 14.58$, $P < 0.05$). The slope of linear trend was –0.12 and was significant (Figure 1). The mean urinary fluoride level in March 2014 was 1.8 ± 0.35 and that in November 2014 was 0.69 ± 0.13 (Table 2). In 11 out of 27 test subjects (serial nos 1–9, 12 and 13), the urinary fluoride level reduced to around 0.1 mg/l in the ninth month of dietary intervention, indicating relief due to consumption of amla powder in addition to defluoridated water. In these individuals there was 72% reduction urinary fluoride level due to dietary intervention. In another 11 subjects (serial nos 17–27), there was 45% reduction and in 5 subjects

Table 1. Impact of consumption of safe water and amla on health complaints of fluorotic subjects of Bhoopnagar village, Gaya district, Bihar

Health effects	Percentage of respondents affected before dietary intervention	Percentage of recovery after 9 months
Gastrointestinal problems	100	60
Joint pain	56	80
Headache and insomnia	52	57

Table 2. Urinary F level (mg/l) in the fluorotic (test) subjects fed with amla supplement

Respondent	March 2014	April 2014	May 2014	June 2014	July 2014	August 2014	September 2014	October 2014	November 2014
1	0.444	0.362	0.265	0.188	0.188	0.154	0.136	0.112	0.068
2	0.486	0.303	0.155	0.167	0.164	0.118	0.115	0.075	0.066
3	0.538	0.386	0.254	0.211	0.212	0.168	0.128	0.083	0.089
4	0.506	0.43	0.327	0.233	0.234	0.234	0.211	0.151	0.101
5	0.403	0.316	0.281	0.281	0.278	0.223	0.213	0.188	0.119
6	0.221	0.114	0.103	0.104	0.104	0.103	0.084	0.084	0.072
7	0.418	0.301	0.266	0.227	0.223	0.175	0.135	0.102	0.059
8	0.466	0.288	0.27	0.218	0.22	0.189	0.166	0.122	0.088
9	0.408	0.274	0.215	0.215	0.215	0.2	0.184	0.181	0.153
10	3.22	3.01	2.64	2.13	2.1	1.88	1.65	1.61	1.36
11	8.11	7.2	6.76	5.27	4.78	4.11	3.79	3.42	3.02
12	2.231	2.06	1.87	1.03	0.887	0.712	0.649	0.644	0.652
13	1.24	1.12	1.03	0.886	0.883	0.645	0.644	0.619	0.586
14	3.31	3.11	2.87	2.35	2.1	1.85	1.48	1.22	1.22
15	6.22	5.67	5.45	4.86	4.61	3.22	2.81	1.97	1.73
16	4.11	3.66	3.59	3.25	3.03	2.83	2.35	2.31	1.88
17	1.56	1.26	1.13	1.04	0.934	0.756	0.661	0.578	0.555
18	2.24	2.21	2.14	1.77	1.27	1.14	1.02	0.935	0.858
19	0.762	0.662	0.631	0.622	0.616	0.602	0.568	0.517	0.511
20	1.02	0.924	0.891	0.835	0.776	0.689	0.691	0.611	0.541
21	0.938	0.886	0.836	0.775	0.725	0.711	0.716	0.721	0.722
22	0.875	0.803	0.762	0.657	0.641	0.614	0.528	0.519	0.518
23	1.32	1.06	0.957	0.922	0.821	0.833	0.737	0.686	0.634
24	1.487	1.22	1.16	1.11	1.05	1.42	1.25	1.17	0.964
25	1.674	1.287	1.19	0.978	0.925	0.861	0.823	0.823	0.833
26	0.866	0.843	0.805	0.773	0.714	0.883	0.764	0.644	0.602
27	0.876	0.792	0.783	0.756	0.721	0.879	0.845	0.822	0.788

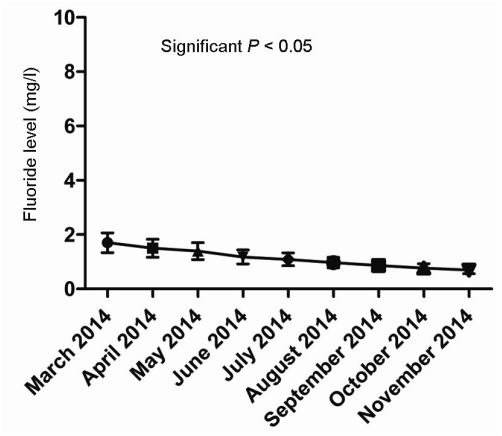

Figure 1. Urinary F level in fluorotic (test) subjects fed with amla supplement.

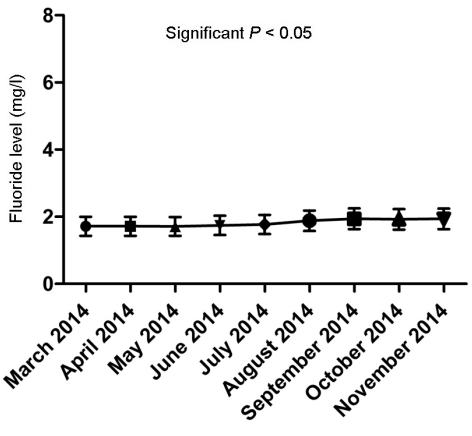

Figure 2. Urinary F level in fluorotic (control) subjects without amla supplement.

(serial nos 10, 11, 14–16), there was 63% reduction in the urinary fluoride level from March to November 2014, although it was above the normal reference range. This is evident from the slope of the linear trend shown in Figure 1. These individuals also should have had some relief from the effect of fluorosis.

The above observations are in contrast to the data reported in Table 3. There was significant rise in the mean fluoride level in the urine of control subjects ($F = 5.27$, $P < 0.05$). The slope of the linear trend was 0.03 and was also significant (Figure 2). The mean urinary fluoride level in March 2014 was 1.7 ± 0.28 and that in November 2014 was 1.9 ± 0.3 (Table 3). In 13 out of 26 control group individuals, the urinary fluoride level was below 1.0 mg/l in November 2014, but in none of them it reached the normal limit of less than 0.1 mg/l. The remaining 13 control subjects had high urinary fluoride level (Table 3) ranging from 1.19 to 6.33 mg/l, as also evident from the positive trend shown in Figure 2.

The test group, fed on amla powder, showed reduction in their urinary fluoride level from 0 to 9 months, although both the test and control groups consumed the

Table 3. Urinary F level (mg/L) in fluorotic (control) subjects without amla supplement

Respondent	March 2014	April 2014	May 2014	June 2014	July 2014	August 2014	September 2014	October 2014	November 2014
1	0.858	0.858	0.858	0.854	0.894	0.895	0.887	0.901	0.922
2	0.865	0.866	0.865	0.934	0.923	0.923	0.93	0.923	0.92
3	0.823	0.827	0.825	0.823	0.877	0.889	0.887	0.887	0.917
4	0.892	0.887	0.888	1.22	1.25	1.33	1.29	1.25	1.19
5	0.875	0.875	0.875	0.884	0.944	0.951	0.967	0.958	0.966
6	0.838	0.841	0.844	0.812	0.811	0.81	0.835	0.844	0.811
7	0.673	0.677	0.676	0.675	0.675	0.669	0.726	0.729	0.722
8	0.786	0.782	0.783	0.788	0.784	0.795	0.795	0.789	0.815
9	0.883	0.884	0.881	0.922	0.924	1.12	0.977	0.977	0.977
10	0.788	0.788	0.788	0.794	0.821	1.06	1.16	1.18	1.23
11	2.3	2.36	2.33	2.32	2.41	4.44	4.51	4.48	4.43
12	1.86	1.8	1.76	1.86	1.86	1.92	1.97	1.95	1.97
13	0.92	0.92	1.08	1.12	1.25	1.25	1.34	1.33	1.42
14	0.88	0.87	0.827	0.826	0.827	0.826	0.874	0.874	0.876
15	4.81	4.83	4.88	4.87	4.89	4.88	4.85	4.84	4.83
16	6.21	6.19	6.2	6.42	6.4	6.35	6.46	6.27	6.33
17	2.84	2.81	2.88	2.93	3.01	3.11	3.62	3.57	3.56
18	2.8	2.86	2.79	2.75	2.75	2.78	2.74	2.76	2.91
19	3.24	3.21	3.18	3.18	3.22	3.44	3.61	3.64	3.66
20	3.66	3.68	3.55	3.54	3.68	3.65	3.79	3.66	3.71
21	0.865	0.863	0.795	0.812	0.833	0.842	0.846	0.855	0.841
22	0.776	0.782	0.689	0.661	0.662	0.671	0.699	0.679	0.716
23	1.32	1.331	1.36	1.36	1.36	1.37	1.45	1.48	1.48
24	0.735	0.733	0.742	0.741	0.74	0.751	0.766	0.759	0.761
25	0.821	0.83	0.88	0.881	0.923	0.921	0.93	0.941	0.955
26	2.23	2.214	2.21	2.35	2.3	2.28	2.45	2.47	2.41

same defluoridated water. Gastrointestinal problems were found to improve in the test subjects who took amla as dietary supplement. Amla has been found to have healing effects on ulcer[28] and it also acts as a laxative[29].

The results of the present study suggest that the supplementation of diet with amla powder and also consumption of safe water (fluoride level not exceeding 1 mg/l) may help alleviate the effect of fluoride toxicity as well as fluorosis. Amla is well known for its nutritional qualities. It is rich in tannins, flavonoids, polyphenols and minerals, and is regarded as one of the richest sources of vitamin C, containing 200–900 mg per 100 g of edible portion[30]. The supplementation of ascorbic acid has been observed to markedly reduce fluoride retention in the bones of mice[15]. Vitamin C supplementation may help reduce the severity of bone fluorosis, because it helps in the synthesis of intercellular cementing substance responsible for keeping the cells of the body together[31]. Therefore, fluorosis could be controlled by creating awareness among the villagers about the importance of fluoride-free water and the inclusion of amla as a dietary supplement.

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Effects of grassland degradation on air and soil temperature in Songnen plain of northeast China

Xiangjin Shen[1,2], Binhui Liu[3] and Daowei Zhou[1,*]

[1]Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Changchun 130102, China
[2]University of Chinese Academy of Science, Beijing 100049, China
[3]College of Forestry, Northeast Forestry University, Harbin 150040, China

Grassland degradation due to human activities is a critical problem in Songnen plain of northeast China, but the possible regional climate changes, especially air and soil temperature changes because of grassland degradation, are still not clearly known. From May to September of 2014, we simulated a *Leymus chinensis* degradation experiment in Songnen plain to study the effects of grassland degradation on air temperature, soil temperature and the difference between soil and air temperatures. The results demonstrated that grassland degradation will increase both the growing season surface air and soil temperature by 0.49–1.49°C and 0.6–2.27°C respectively. The difference between soil and air temperatures also increases with the reduction of *L. chinensis* vegetation cover, implying that the increase in soil temperature will be much faster than air temperature with the degradation of the grassland in Songnen plain.

Keywords: Climate change, grassland degradation, *Leymus chinensis*, surface air temperature, soil temperature.

IN the background of global warming, both surface air and soil temperatures have increased rapidly in the last few decades[1,2]. The increase in air and soil temperatures will have a significant effect on plant growth by influencing the growth of overground and underground parts. Vegetation changes, in turn, could also change surface air and soil temperatures by affecting the land surface water and energy balance[3]. Studying the effects of vegetation change on air or soil temperature is critical for understanding the interactions between vegetation and climate[4,5], and becomes an interdisciplinary research effort[6].

Due to human disturbances, grasslands in arid and semi-arid regions of North China are facing degradation[7]. Grassland degradation not only affects the carbon cycle and regional economy, but also has a significant impact on climate[8]. Many researchers have studied the effect of grassland degradation on evapotranspiration[9–11] and surface water balance[12] in these regions of North China. However, the effects of grassland degradation on surface temperature, especially soil temperature, have received little attention.

*For correspondence. (e-mail: zhoudaowei@neigae.ac.cn)