TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2013) 37: 529-534
© TÜBİTAK
doi:10.3906/vet-1302-2

The levels of trace elements and selected vitamins in goats with chronic fluorosis

Nuri ALTUĞ[1,*], Sezai ARSLAN[2], Nazmi YÜKSEK[3], İhsan KELEŞ[4], İbrahim Hakkı YÖRÜK[5], Yıldıray BAŞBUĞAN[3], İsmail AYTEKİN[6]

[1]Department of Internal Medicine, Faculty of Veterinary Medicine, Kırıkkale University, Kırıkkale, Turkey
[2]Department of Pathology, Pendik Veterinary Control and Research Institute, İstanbul, Turkey
[3]Department of Internal Medicine, Faculty of Veterinary Medicine, Yüzüncü Yıl University, Van, Turkey
[4]Department of Internal Medicine, Faculty of Veterinary Medicine, Erciyes University, Kayseri, Turkey
[5]Department of Chemistry, Faculty of Arts and Sciences, Yüzüncü Yıl University, Van, Turkey
[6]Department of Internal Medicine, Faculty of Veterinary Medicine, Balıkesir University, Balıkesir, Turkey

Received: 01.02.2013 • **Accepted:** 17.04.2013 • **Published Online:** 26.08.2013 • **Printed:** 20.09.2013

Abstract: This study was conducted to investigate the effects of chronic fluorosis on trace elements and vitamin levels in goats. Thirty-three goats exhibiting clinical signs of chronic fluorosis and 10 healthy goats from the same region were used. Animals with chronic fluorosis were divided into 2 groups according to their age as 1–3 years old and 4–6 years old. Blood samples were collected from all goats. Fluoride concentrations were determined with an ionometer. Na, K, and Cl levels were measured with ion-selective equipment and serum mineral substance levels were obtained by atomic absorption spectrophotometer. Ca, Mg, and P levels were determined by biochemistry analyzer. Serum vitamin concentrations were determined by liquid chromatography. The levels of serum copper, iron, manganese, and nickel decreased in goats with chronic fluorosis, whereas other trace elements did not change. Among vitamins, levels of serum α-tocopherol were increased, whereas retinol and vitamin D3 levels were not changed significantly differently between the fluorosis and control groups. In evaluation of goats with fluorosis according to age group, copper levels in the age group of 1–3 years and iron levels in the age group of 4–6 years were found to be lower than in the other age groups. Other minerals and all vitamin parameters were not different according to age. The results of the present study clearly showed that serum copper, iron, manganese, and nickel levels were reduced in goats with chronic fluorosis, and so goats with fluorosis should receive support in terms of these concerned trace elements.

Key words: Fluorosis, goat, trace elements, vitamins

1. Introduction

Fluorosis is a chronic poisoning from long-term exposure to a high level of fluoride, and it is a serious problem in ruminants (1). The problem is endemic in many countries in those areas where the F contents are high in drinking waters (1–3). Volcanic areas in the world are especially rich in F. High levels of F in drinking water have been documented in Turkey, especially in the large areas located around Tendürek Volcanic Mountain (3,4).

Lesions in chronic fluorosis are characteristic in teeth and bones, and the signs are largely referable to these lesions. Because F alters the absorption of some elements from the gastrointestinal tract and also binds with some of elements in the body such as Ca, Mg, and Mn (1,5), excessive F in the body leads to deficiencies in the normal levels of some elements (5–8). Fluorides inhibit the action of ameloblasts and odontoblasts during tooth formation, resulting in failure of the developing tooth to accept minerals. In bones, fluorides alter mineralization and remodeling of bone by replacing hydroxyapatite in the bone crystalline structure. Additionally, locomotor function and nutrition may be disrupted due to reduced feed intake (9,10). In addition, Bouaziz et al. (11) reported that excessive F can induce a decrease in serum levels of nonenzymatic antioxidants such as some dietary minerals such as zinc and copper, known to be cofactors of superoxide dismutase.

Studies show that F ions play a role in the free-radical processes (12). Although the relationship in both human and animal fluorosis among free radical generation, lipid peroxidation, and antioxidant defense systems has been investigated extensively, these various studies have produced conflicting results (12). Vitamins A, C, and E help protect the organism from free radical oxidation in fluorosis (13). In addition, vitamin D plays a role in the amelioration of fluoride-induced toxicity (14). Various

* Correspondence: nurialtug@kku.edu.tr

researchers have also reported a relationship between F and vitamins (9,15–17) and between vitamins and the treatment of fluorosis (9–11). However, studies in this respect in ruminants are very limited (8).

Trace elements in sheep (5,8,18) and cattle (19,20) with fluorosis have been reported by researchers, but there are very limited studies about the effects of fluorosis on trace element levels in goats (2,6). Moreover, some trace elements (Zn, Cu, Fe, Mn, Pb, Ni, Cd) and vitamin levels in goats with fluorosis could not be cited from the literature. Therefore, we investigated the effects of chronic fluorosis on mineral and vitamin levels in goats.

2. Materials and methods

2.1. Animal materials and study design

This study was conducted on 33 goats exhibiting clinical signs of fluorosis from villages in the Tendürek Mountain Volcanic Region of Turkey. Clinical symptoms observed were softening and discoloration of teeth, corrosion of teeth, difficulty in chewing, lameness, bone exostoses, and nail deformities. All animals used in this study were aged between 1 and 6 years old and were of the same breed. According to the age, goats with fluorosis were divided into 2 groups as 1–3 years and 4–6 years. None of these goats received drugs for prevention from fluorosis. For the control, 10 goats from the same region, but from a different village, were also used. Clinical findings of fluorosis were not observed in these control animals. Breeding conditions, nutrition, and source of drinking water were identical in both groups, with the exception of blood F levels.

2.2. Collection of samples

Blood samples in all goats were obtained from the v. jugularis puncture and were collected into sterile tubes without anticoagulant. After clotting for 1 h at room temperature, blood samples were centrifuged (Rotofix 32, Hettich) at 4000 × *g* for 10 min at room temperature and sera were carefully harvested and stored at –20 °C until biochemical analysis.

2.3. Determination of fluoride

Fluoride concentrations of serum were determined with an ionometer (Orion 720 A) using a fluoride-selective ion electrode (Orion 96 09 00 BN).

2.4. Analysis of mineral substances

Serum sodium, potassium, and chloride levels were measured with ion-selective equipment (ISE, Medica). Serum mineral substance levels, including those of zinc, copper, iron, manganese, lead, nickel, and cadmium, were determined using an atomic absorption spectrophotometer (Solar AA Spectrometers, Thermo Scientific). Serum calcium, magnesium, and phosphorus levels were determined by automated biochemistry analyzer (Roche Hitachi 912).

2.5. Measurement of serum vitamin levels

The quantitative analysis of serum vitamin A, D, and E levels in the samples was conducted by high performance liquid chromatography (Agilent 1100 Series) according to the procedure modified from the relevant literature (21,22).

2.6. Statistical analysis

For statistical analysis, the independent samples t-test was used to determine the difference between the values obtained from the control and fluorosis groups. Statistical evaluation of differences between goats aged 1–3 and 4–6 was done using the paired samples t-test. For this purpose, SPSS 16.0 was used. Statistical significance was set at $P < 0.05$. All data were expressed as means ± standard error of the means (SEM).

3. Results

Changes in F levels are presented in the Figure. There were significant increases ($P < 0.001$) in serum F levels in the fluorosis groups compared to the control group, but these parameters were not different statistically when age groups were compared.

Changes in mineral substances are presented in Table 1. There were significant decreases in serum Cu, Fe, Mn, and Ni levels in the fluorosis group compared to the control group. However, other parameters (Na, K, Cl, Ca, P, Mg, Zn, Pb, Cd) did not change significantly. Although Fe, Mn, and Ni levels were also decreased in both age groups compared to the control group, Cu levels decreased only in the fluorosis group of 1–3 years old. When evaluations were made according to age group in the fluorosis groups, Cu levels in the 1–3 age group and Fe levels in the 4–6 age group were found to be lower than in the other age groups (Table 1).

Changes in vitamin levels are presented in Table 2. There were significant increases in α-tocopherol levels

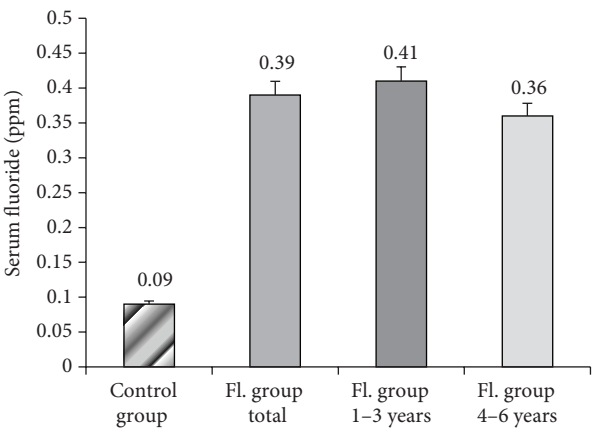

Figure. Serum fluoride levels in the control group and fluorosis (Fl.) groups. Results are expressed as mean ± SEM.

Table 1. Serum mineral substance levels of goats with fluorosis and the control group.

Parameters	Control group Mean ± SEM (n = 10)	Fluorosis group Mean ± SEM		
		Total (n = 33)	1-3 years (n = 22)	4-6 years (n = 11)
Na (mEq/L)	142.1 ± 11.5	146.7 ± 12.8	148.2 ± 13.4	143.7 ± 14.2
K (mEq/L)	5.30 ± 0.55	4.42 ± 0.52	4.36 ± 0.50	4.54 ± 0.54
Cl (mEq/L)	102.3 ± 11.1	102.6 ± 11.3	103.1 ± 11.9	101.6 ± 12.3
Ca (mg/dL)	8.85 ± 0.58	8.66 ± 0.33	8.97 ± 0.29	8.04 ± 0.37
P (mg/dL)	5.13 ± 0.87	4.78 ± 0.60	4.87 ± 0.48	4.60 ± 0.73
Mg (mg/dL)	2.62 ± 0.10	2.31 ± 0.16	2.35 ± 0.18	2.23 ± 0.21
Zn (mg/L)	0.574 ± 0.08	0.603 ± 0.03	0.623 ± 0.034	0.562 ± 0.056
Cu (mg/L)	0.550 ± 0.05	0.481 ± 0.03^{b}	0.429 ± 0.04^{c***}	0.584 ± 0.06
Fe (mg/L)	1.245 ± 0.03	1.099 ± 0.05^{b}	1.121 ± 0.07^{a}	1.055 ± 0.08^{b*}
Mn (mg/L)	0.0186 ± 0.02	0.0167 ± 0.01^{a}	0.0166 ± 0.01^{a}	0.0168 ± 0.02^{a}
Pb (mg/L)	0.109 ± 0.03	0.109 ± 0.02	0.110 ± 0.02	0.107 ± 0.03
Ni (mg/L)	0.166 ± 0.01	0.138 ± 0.02^{a}	0.146 ± 0.02^{a}	0.122 ± 0.04^{a}
Cd (mg/L)	0.023 ± 0.02	0.022 ± 0.01	0.021 ± 0.01	0.023 ± 0.01

Statistical importance between control and fluorosis groups: $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$.
Statistical importance between the ages of 1–3 and 4–6: $^{*}P < 0.05$, $^{***}P < 0.001$.

Table 2. Serum vitamin levels of goats with fluorosis and control group.

Parameters	Control group Mean ± SEM (n = 10)	Fluorosis group Mean ± SM		
		Total (n = 33)	1–3 years (n = 22)	4–6 years (n = 11)
Retinol (µg/mL)	0.588 ± 0.045	0.686 ± 0.024	0.678 ± 0.027	0.702 ± 0.050
α-Tocopherol (µg/mL)	1.282 ± 0.066	1.711 ± 0.078^{b}	1.702 ± 0.096^{b}	1.729 ± 0.138^{b}
Vitamin D_3 (µg/mL)	0.0134 ± 0.0008	0.0140 ± 0.0006	0.0144 ± 0.0007	0.0132 ± 0.0012

Statistical importance between control and fluorosis groups: $^{b}P < 0.01$.

in the fluorosis groups compared to the control group. However, these parameters were not different when age groups were compared. Retinol and vitamin D_3 levels were not different significantly between the fluorosis and control groups (Table 2).

4. Discussion

Serum F levels in the control group were within normal limits, whereas they were very high in goats with fluorosis (Figure). In the present study we did not evaluate water F levels. However, in a previous study conducted in the

same region (4), F concentrations in the drinking water from these villages with fluorosis and from the control area were reported to be 9.5–13.7 ppm and 0.16–0.53 ppm, respectively. The maximum permissible limit of F levels in drinking water has been indicated as 1.5 ppm by the World Health Organization (23). Thus, if breeding conditions, nutrition, and source of drinking water are identical in both groups, one can assume that the biological effects observed are mainly due to fluoride.

Changes of serum mineral substance in fluorosis have been reported in human beings (24) and animals (3,5,6,16,18). Different results depending on animal species, and natural or experimental conditions were reported with concern to mineral level (2,5–7,20). In the present study, Cu, Fe, Mn, and Ni levels in serum of goats with fluorosis were significantly decreased compared to the control group (Table 1). However, levels of other mineral substances such as Na, K, Cl, Ca, P, Mg, Zn, Pb, and Cd did not change. Moreover, Cu, Zn, Fe, Mn, Pb, Cd, and Ni levels were detected for the first time in this study in goats with fluorosis according to our knowledge of the literature.

The decreased levels of serum Cu in the present study were similar to the findings reported in sheep (18), bovine (20), and humans (24) with fluorosis. Cenesiz et al. (25) reported that serum uric acid levels were increased in sheep with fluorosis. Uric acid has a protective role in the defense mechanisms of the body and is also a lipid peroxidation inhibitor and a radical scavenger (26). Uric acid is tightly bound to copper and iron, both of which are known to have important roles in antioxidative defense mechanisms (27). Therefore, the decrease in serum Cu and Fe in the present study might be the result of the radical removing reactions that are produced by F. Serum copper levels in the group of 1–3 years old were found to be lower than in the control and the 4–6 age group. This situation may be explained by the idea that copper deficiency induced by fluorosis may have been tolerated with advancing age.

It is known that high fluoride ingestion disturbs the metabolism of some metals in animals and humans beings (9). As with Cu levels, similar decreases in Fe, Mn, and Ni levels were also determined in both fluorosis groups in our study. This finding is in agreement with the results of Tao et al. (7) except for Zn, as they reported that animals exposed to excessive fluoride had decreases in Zn levels (7). Decreased Fe levels in the present study may also have resulted from the decrease in Fe incorporation in the blood with a concomitant increase in Fe uptake in the bone marrow and the liver, which was similarly reported by Kahl et al. (28). In addition, decrease in Fe levels in this study may be explained by the above statements regarding reductions in the levels of Cu.

It was suggested that excessive fluoride ingestion disturbs manganese metabolism (29). In this context, it was reported that serum Mn levels decreased in pigs (7) and in humans (24), whereas it increased in cattle (20). In the present study, serum Mn levels significantly decreased in goats with fluorosis. This finding was in agreement with some other studies' findings (7,24). Decreased serum levels of Mn in the present study can be explained by the conclusions of Li et al. (30), who speculated that the formation of insoluble complexes with F in the gastrointestinal tract probably affected the retention of Mn in the body. However, the interaction between Mn levels and excessive fluoride ingestion requires further investigations.

In the present study, serum Ni levels were low in goats with fluorosis. Serum Ni levels in ruminants with fluorosis could not be cited from the literature, but rumen bacterial urease has been shown to be a Ni-dependent enzyme and Ni is a component of factor F_{430}, present in methanogenic bacteria (31). Furthermore, researchers reported that serum urea concentrations increased (2,5,20) while total protein levels decreased in animals with fluorosis (2,5). Moreover, Bennis et al. (2) explained that higher urea concentrations could be related to restricted food intake, which determines an increase of protein breakdown and thus of urea excretion. When considering the above points (2,5,20,31), the decreased Ni levels in the present study might be speculated to be connected with hypoproteinemia. Hypoproteinemia can cause a negative energy balance such that ruminal degradation of urea can be increased, ultimately increasing the usage of urease, which may cause a decrease in the Ni level.

In this study, alterations of minerals (Ca and P) and vitamin D related to bone metabolism were not observed. Similar findings with concern to levels of serum Ca were also determined in previous studies in goats with fluorosis (2,6). Serum P levels in ruminants with fluorosis were evaluated in the same context. However, some researchers observed an increase (2,23) while others reported no changes (5,6,19) in serum P levels. Serum vitamin D levels in ruminants with fluorosis were measured for the first time in this study according to our knowledge of the literature, and they were not changed significantly according to our findings. These findings are consistent with the findings of an experimental study in pigs (15). In the present study, adequate levels of vitamin D were determined, which facilitates absorption of Ca from the gastrointestinal tract. Similar findings were reported in experimental studies by other researchers (14,15). However, the same researchers reported that disturbance of Ca homeostasis is not a necessary finding in dental and skeletal fluorosis and consequently vitamin D does not prevent the deleterious effects of F on motor coordination and teeth.

Studies on vitamin levels during exposure to fluoride are limited and results are conflicting. Vatassery et al. (17)

observed elevated levels of α-tocopherol in serum during the chronic phase of fluoride intoxication, but Yasar and Yur (8) did not detect any significant change in vitamin E levels in sheep with fluorosis. In the present study, serum α-tocopherol levels in goats with chronic fluorosis were found to be increased. Our findings are in accordance with those of Vatassery et al. (17). Although α-tocopherol is an antioxidant vitamin, its level in this study was increased. There are many studies about the relationship between oxidative stress and fluorine. In this context, there are also studies disclosing the activation (11), inhibition (13), and lack of effects (16) of F ions on lipid peroxidation. Chlubek et al. (13) suggested that fluoride at relatively low concentrations stimulates lipid peroxidation, but at high and very high concentrations it may act as an inhibitor of malondialdehyde generation. There was no reduction in the α-tocopherol levels in the present study. This situation may be explained by the reports of Chlubek et al. (13). However, the mechanism of increased α-tocopherol levels in chronic fluorosis cannot be explained without additional studies on the absorption, transport, and tissue deposition of α-tocopherol.

Serum retinol levels in the present study did not change. In contrast, Yasar and Yur (8) reported that serum vitamin A and β-carotene levels decreased in sheep with fluorosis. According to same authors, decreased levels in serum vitamin A and β-carotene could be caused by the reduced food intake and oxidative stress caused by the toxicity of F. However, we do not agree with them fully because vitamin A levels in animal nutrition is directly related to the nutrition season, ration, and nutrient supplementation. Similarly, Wang et al. (32) reported that fluorosis is related to nutrition season and nutrient supplementation. Moreover, some authors (12,33) proposed that the ages, animal species, time of exposure, medications, daily consumption of water, and fluoride levels in the blood should also be taken into consideration in cases of fluorosis. Therefore, changes in the levels of vitamin A in animals with fluorosis may be due to the different factors mentioned by these researchers (12,32,33).

In conclusion, this study clearly shows that serum Cu, Fe, Mn, and Ni levels were reduced in goats with chronic fluorosis, and so goats with fluorosis should receive support in terms of these mineral substances. The secondary results of the present study are that evaluations of the level of serum Ca and vitamin D_3 in goats with fluorosis may be useful, but the serum Ca levels may not be directly linked to dental and bone disorders. Although α-tocopherol and retinol are antioxidant vitamins, α-tocopherol levels increased in this study and retinol levels did not change. Therefore, to interpret such cases more accurately, changes in levels of vitamins in ruminants with fluorosis must be evaluated together with several factors such as differences in nutritional season, ration, and absorption, transport, and tissue distribution of the vitamins.

References

1. Choubisa SL. Some observations on endemic fluorosis in domestic animals in Southern Rajasthan (India). Vet Res Commun 1999; 23: 457–465.
2. Bennis A, Kessabi M, Hamliri A, De La Farge F, Braun JP. Plasma biochemistry of adult goats wıth chronic fluoride poisoning in morocco. Fluoride 1993; 26: 241–246.
3. Oruc N. Occurrence and problems of high fluoride waters in Turkey: an overview. Environ Geochem Health 2008; 30: 315–323.
4. Arslan S. The relationship between thyroid hormones and fluoride levels in goats with fluorosis. PhD, Yüzüncü Yıl University Institute of Health Science, Van, Turkey, 2008 (in Turkish).
5. Kessabi M, Boudarine B, Braun JP, Lamnaouer D. Serum biochemical effects of fluoride in sheep of the Darmous area. Fluoride 1983; 16: 214–219.
6. Kant V, Srivastava AN, Verma PK, Raina R. Alterations in biochemical parameters during subacute toxicity of fluoride alone and in conjunction with aluminum sulfate in goats. Biol Trace Elem Res 2009; 130: 20–30.
7. Tao X, Xu ZR, Wang YZ. Effect of excessive dietary fluoride on nutrient digestibility and retention of iron, copper, zinc and manganese in growing pigs. Biol Trace Elem Res 2005; 107: 141–151.
8. Yasar S, Yur F. Antioxidant vitamin and mineral levels in sheep with fluorosis. Biol Trace Elem Res 2008; 123: 139–143.
9. Cerklewski FL. Fluoride bioavailability nutritional and clinical aspects. Nutr Res 1997; 17: 907–929.
10. Susheela AK, Bhatnagar M. Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. Mol Cell Biochem 2002; 234–235: 335–340.
11. Bouaziz H, Croute F, Boudawara T, Soleilhavoup JP, Zeghal N. Oxidative stress induced by fluoride in adult mice and their suckling pups. Exp Toxicol Pathol 2007; 58: 339–349.
12. Chlubek D. Fluoride and oxidative stress. Fluoride 2003; 36: 217–228.
13. Chlubek D, Zawierta J, Kazmierczyk A, Kramek J, Olszewska M, Stachowska E. Effect of different fluoride ion concentrations on malondialdehyde (MDA) formation in the mitochondrial fraction of human placental cells. Bromat Chem Toksykol 1999; 32: 119–122.

14. Ekambaram P, Paul V. Effect of vitamin D on chronic behavioral and dental toxicities of sodium fluoride in rats. Fluoride 2003; 36: 189–197.

15. Andersen L, Richards A, Care AD, Andersen HMK, Kragstrup J, Fejerskov O. Parathyroid glands, calcium, and vitamin D in experimental fluorosis in pigs. Calcif Tissue Int 1986; 38: 222–226.

16. Reddy GB, Khandare AL, Reddy PY, Rao GS, Balakrishna N, Srivalli I. Antioxidant defense system and lipid peroxidation in patients with skeletal fluorosis and fluoride-intoxicated rabbits. Toxicol Sci 2003; 72: 363–368.

17. Vatassery GT, Ophaug RH, Singer L. The effect of fluoride intake on the total lipid, cholesterol and vitamin E levels in sera and liver of guinea pigs on high fat diet. Life Sci 1980; 27: 1961–1966.

18. Maraşlı Ş, Maraşlı N, Yenigün A. The preliminary report on hypocupraemia in sheep with enzootic fluorosis. Kafkas Univ Vet Fak Derg 1995; 1: 79–81 (article in Turkish with an English abstract).

19. Kapoor V, Prasad T, Paliwal VK. Blood biochemical constituents in calves following subclinical levels of fluoride toxicosis. Fluoride 2001; 34: 126–131.

20. Singh JL, Swarup D. Biochemical changes in serum and urine in bovine fluorosis. Indian J Anim Sci 1999; 69: 776–778.

21. Miller KW, Yang CS. An isocratic high-performance liquid chromatography method for the simultaneous analysis of plasma retinol, α-tocopherol and various carotenoids. Anal Biochem 1985; 145: 21–26.

22. Reynolds SL, Judd HJ. Rapid procedure for the determination of vitamins a and d in fortified skimmed milk powder using high-performance liquid chromatography. Analyst 1984; 109: 489–492.

23. WHO. Environmental Health Criteria for Fluorosis. Geneva: World Health Organization; 1984.

24. Meral I, Demir H, Gunduz H, Mert N, Doğan I. Serum copper, zinc, manganese, and magnesium status of subjects with chronic fluorosis. Fluoride 2004; 37: 102–106.

25. Cenesiz S, Ozcan A, Kaya N, Baysu N, Karabulut AB. Chronic effects of fluoride in Tuj sheep on serum levels of total protein, albumin, uric acid, and nitric oxide and activities of lactate dehydrogenase and leucine aminopeptidase. Fluoride 2005; 38: 52–56.

26. Yu BP. Cellular defenses against damage from reactive oxygen species. Physiol Rev 1994; 74: 139–162.

27. Cross CE, Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. Ann Intern Med 1987; 107: 526–545.

28. Kahl S, Wolcik K, Ewy Z. Effects of fluoride on some hematological indices and Fe distribution in blood and iron storing tissues in rat. Bull Acad Pol Sci Ser Sci Biol 1973; 21: 389–393.

29. Singh M. Effect of fluoride on tissue manganese levels in the mouse. Sci Total Environ 1982; 22: 285–288.

30. Li CS, Tan YB, Zhang LL. The recognition of fluorosis as a chemical question through trace element analysis of the liver and spleen of the monkey. Endemic Dis Bull 1990; 5: 1–5.

31. Spears JW. Nickel as a "newer trace element" in the nutrition of domestic animals. J Anim Sci 1984; 59: 823–835.

32. Wang JD, Hong JP, Li JX. Studies on alleviation of industrial fluorosis in Baotou goats. Fluoride 1995; 28: 131–134.

33. Chinoy NJ. Fluoride stress on antioxidant defense systems. Fluoride 2003; 36: 138–141.