EFFECTS OF *PANAX GINSENG* ON FLUORIDE-INDUCED HAEMATOLOGICAL PATTERN CHANGES IN MICE

Ali Karadeniz,[a] Levent Altintas[b]

Erzurum and Ankara, Turkey

SUMMARY: Various effects of *Panax ginseng* (ginseng) on changes in haematological parameters induced by fluoride (F) in mice were studied. Sixty male adult Swiss albino mice were divided into six groups of ten mice in each group. F was supplied at a concentration of 40 mg F ion/L in the drinking water to group I without ginseng and to groups II and III along with ginseng at a level of 100 and 200 mg/kg bw/day, respectively. Groups IV and V were supplied with ginseng only, and group VI served as the control without F or ginseng. Blood samples were taken on days 0, 15, and 30 from the hearts of the mice, and the counts of red blood cells (erythrocytes, RBC), white blood cells (leucocytes, WBC), neutrophils, and lymphocytes, and the levels of haemoglobin and haematocrit were determined. Although the lymphocyte count increased slightly, by the 30th day in the F group I a statistically significant decrease was observed in RBC, WBC, and neutrophil counts, as well as the haemoglobin and haematocrit values. On the other hand, in the F plus ginseng groups II and III, a restorative increase comparable to levels in the control group was found by the 15th and 30th day in these parameters, respectively, in the RBC, WBC, neutrophil, and lymphocyte counts and the haemoglobin and haematocrit values. These results indicated that 40 mg F/L in the drinking water caused anemia in mice and that *Panax gensing* counteracted this effect.

Keywords: Anemia; Fluorosis in mice; Ginseng; Haematology in mice; *Panax ginseng*.

INTRODUCTION

Fluoride (F) is a major environmental contaminant, the impact of which on various human and animal tissues and organs has been studied extensively.[1,2] With excessive ingestion of F, chronic fluoride intoxication or fluorosis occurs.[3] Adverse hematological effects of F have also been reported including damage to hematopoietic organs.[4-7]

For over 2000 years ginseng root has been used in the belief that it is a panacea and promotes longevity.[8] The predominant physiologically active components in ginseng are triterpenoid saponin glycosides, also known as ginsenosides, and at least 25 ginsenosides have been identified from Asian ginseng.[9] Pharmacological effects of ginseng have been demonstrated in the central nervous system and in cardiovascular, endocrine, and immune systems.[10] For example, it has been reported that ginsenosides increase humoral and cell mediated immune responses,[11] increase the number of antigen-reactive T helper cells and T lymphocytes,[12] and inhibit tumor angiogenesis and metastasis.[13] However, we have not found any reports on the use of ginseng to treat fluorosis. In this work, as an extension of recent results in our laboratory on the effects ginseng on peripheral blood cells in rats,[14] protective effects of ginseng on some haematological parameters in mice ingesting F in their drinking water were investigated.

[a]For Correspondence: Department of Physiology, Faculty of Veterinary Medicine, University of Atatürk, 25700/Erzurum, Turkey. E-mail: karadenizali@gmail.com. [b]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Ankara, 06110/ Ankara, Turkey. E-mail:laltintas@hotmail.com.

MATERIALS AND METHODS

Source of chemicals and Panax ginseng: Sodium fluoride (NaF) was purchased from Merck (Art No: 6441). The ginseng (*Panax ginseng*) was obtained from Sağlık ve Güzellik Merkezi, Ankara, Turkey.

Animals and housing: Sixty adult male Swiss albino mice were divided into six groups of 10 mice in each group. Each group of animals was housed in stainless steel cages measuring 360×200×190 mm during the study under standard laboratory conditions (21±2ºC and 55% relative humidity and light period 07.00 to 20.00 hr). The mice received humane care according to the criteria outlined in the *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences and published by the National Institute of Health. All experiments were approved by the Ethical Committee of the Atatürk University.

The determined doses of F and ginseng were applied by adding them to the drinking water. At the beginning of the study, counted as day zero, blood was taken from each mouse to determine and compare the RBC (red blood cell) and WBC (white blood cell) values among all six groups. Subsequently, on days 15 and 30 of the experiment, blood was taken again from each animal. RBC and WBC were counted by the haemocytometric method, the haemoglobin level was determined spectrophotometrically, the haematocrit volume was determined by the microhaemotocrit tube method, and percentage rates of neutrophil and lymphocyte were determined by the May-Grunwald-Giemsa staining method.[15]

Experimental design: A stock solution of 5.0 g F ion/L was prepared according to the method of Akdogan et al.[16] by dissolving 11.05 g NaF in one liter of drinking water, preserved at 4ºC and renewed each week. Drinking water for the experimental groups containing 40 mg F^-/L was prepared by a 1:125 dilution of this stock solution. Designations and treatments of the six groups of mice for 30 consecutive days are shown in Table 1.

Table 1. Group designations and experimental protocol (n=10 mice/group)

Group	Designation	Application
I	F	Fluoride (40 mg/L)
II	F + G_1	Fluoride (40 mg/L) + Ginseng (100 mg/kg bw/day)
III	F + G_2	Fluoride (40 mg/L) + Ginseng (200 mg/kg bw/day)
IV	G_1	Ginseng (100 mg/kg bw/day)
V	G_2	Ginseng (200 mg/kg bw/day)
VI	C	Control

All groups were fed the same standard rat pellet diet *ad libitum* (level of F in the pellets unknown). Groups I–III were given the drinking water containing 40 mg F/L, and groups IV–VI were given drinking water containing 0.07 mg F/L. Groups II and IV were given 100 mg ginseng/kg bw (designated G_1), and groups III and V (designated G_2) were given 200 mg ginseng/kg bw. The ginseng was administered orally by adding it to the drinking water. For administration in the drinking water at the two dose levels shown in Table 1, ginseng was dissolved in the drinking water at a concentration of 600 mg/L and 1200 mg/L, respectively. With an average body mass of 30 g and drinking about 5 mL of water per day throughout

the 30-day period of the experiment, the mice were therefore ingesting ginseng at doses of approximately 100 and 200 mg/kg-bw/day

Statistical analysis: The statistical significance of differences between groups was determined by one-way analysis of variance (ANOVA) followed by Tukey post-test using the SPSS 12.0 for Windows. All the values were expressed as means ± S.E.M., and $P < 0.05$ was considered as statistically significant.

RESULTS

Haematological parameters of the six groups of mice and the significance of differences among them are shown on Table 2. In the F Group I, after 30 days the lymphocyte count increased but not significantly, whereas the total RBC count, WBC count, the level of haemoglobin, the haematocrit percentage, and the percentage of neutrophil showed a significant decrease compared to the control Group VI ($P < 0.05$). On the other hand, combined administration of *Panax ginseng* (Group II and Group III) markedly blocked these decreases. In Group IV and Group V without added F, by day 15 ginseng significantly increased the total RBC count, WBC count, the level of haemoglobin, and the haematocrit percentage ($P < 0.05$).

Table 2. Changes in blood parameters resulting from of ginseng application against fluorosis (n=10 mice/group; values ± SEM)

Group and day of sampling	Parameters					
	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Haemoglobin (gr/dl)	Haematocrit %	Neutrophil %	Lymphocyte %
I – F						
0[a]	8.28±0.14	8.80±0.20	14.02±0.44	46.00±1.53	23.00±1.10	73.00±2.60
15	8.07±0.19	10.25±0.45*	11.22±0.17	43.50±1.10	21.50±5.50	75.00±1.20
30	7.40±0.26*	11.05±0.25*	10.56±0.24*	39.00±1.20*	17.00±1.10*	78.50±1.40
II – F+ G_1						
0[a]	8.74±0.67	9.30±2.10	13.14±0.44	42.00±2.05	25.50±3.50	74.50±2.50
15	8.23±0.35	9.34±0.34	12.18±0.44	39.00±1.58	16.00±2.50*	81.50±4.50*
30	8.85±0.40	9.4±1.40	11.28±1.17	41.00±1.76	12.50±3.50*	84.50±5.50*
III – F+ G_2						
0[a]	8.98±0.46	9.35±0.71	12.47±1.22	43.00±1.45	21.00±.3.74	72.00±3.80
15	8.51±1.16	9.57±0.50	12.74±1.43	40.00±1.14	26.50±5.50	75.60±2.40
30	9.18±0.27	9.70±1.05	12.82±0.10	41.00±0.54	20.50±2.40	76.20±2.90
IV – G_1						
0[a]	9.03±0.40	9.25±0.85	13.49±0.38	43.50±0.57	22.00±2.50	72.50±3.50
15	9.57±0.35*	10.28±0.93*	14.10±0.05*	44.40±0.35*	21.00±2.60	77.00±1.25*
30	10.33±0.19*	11.23±0.97*	14.91±0.04*	45.90±0.87*	24.50±0.50	84.00±4.30*
V – G_2						
0[a]	9.58±0.12	8.75±0.33	12.73±0.18	39.50±0.85	18.00±2.45	77.50±2.50
15	10.23±0.03*	10.89±0.81*	13.38±0.05	41.50±0.50	19.00±1.32	79.00±3.10*
30	11.10±0.02*	12.82±2.29*	15.05±0.37*	46.70±0.45*	20.00±2.60	83.00±2.40*
VI – C (control)						
0[a]	9.12±0.79	8.88±0.67	12.30±1.22	42.50±0.35	21.50±2.50	73.50±2.50
15	8.98±0.45	8.95±0.25	12.44±0.41	41.50±0.65	24.50±0.50	72.50±2.50
30	9.28±0.45	9.10±0.85	12.25±0.53	43.50±0.29	23.00±0.70	75.50±3.50

[a]Blood samples collected before treatment. *$p < 0.05$) compared with control group in the same column.

DISCUSSION

In this study, we observed a significant F-associated decrease in RBC and WBC counts, the values of haematocrit, and the levels of haemoglobin as found previously in rats,[17] rabbits,[18] and children[19] exposed to F. Although many aspects of how F affects the body are still unclear, it is known that F can block or induce various changes in blood. The F-induced anemia observed in this study may result from inhibition of globulin synthesis,[20] depression of erythropoiesis, or a decrease in the level of blood folic acid.[21,22] There are many studies of F toxicity on haematological parameters. For example, Machalinski et al.[5] observed that NaF has marked negative effects on hematopoiesis. Choubisa et al.[23] found that in an area of endemic fluorosis, decreased RBC and haemaglobin were present. Hillman et al.[7] reported that cattle afflicted with fluorosis developed anemia and eosinophilia. On the other hand, Uslu[24] did not observe anemia in rats after 45 days of exposure to 30 and 100 ppm fluoride in their drinking water. Also, some studies have shown that NaF stimulates granule formation and oxygen consumption in WBCs, virtually abolishing phagocytosis. Clark[25] found that 1 ppm NaF induced the loss of WBC migration. However, Guo et al.[26] observed that F intoxication affects the immune system, reducing the total WBC count.

As seen here, coadministration of ginseng with F showed beneficial haematological effects. In the ginseng plus F groups II and III, anemia decreased by day 15, and by day 30 the RBC, haemoglobin, and haematocrit values were largely restored. In our view, this protective effect of ginseng against F-induced toxicity can be attributed mainly to its antioxidant ability or its immune system stimulatory effects.[27] In a recent investigation we found that gensing improved the haematological parameters of rats.[14] However, other research indicates that gensing enhances phagocytosis, natural killer cell activity (NK), and the production of interferon while improving the physical and mental performance of mice and rats.[28,29] Kim et al.[30] and Yun et al.[31] report that ginsenoside Rg1 increases both humoral and cell-mediated immune responses. In addition, Kenarova et al.[12] observed that ginsenoside Rg1 increased the number of antigen-reactive T helper cells, T lymphocytes, and NK cells. In addition, although increases in leucocytes, lymphocytes, and alveolar macrophages in gensing-treated animals have been reported,[32,33] such findings are disputed by Srisurapanon et al.[34] Overall, these various findings lead us to conclude that anemia caused by F can be reversed by the antioxidant properties of ginseng and its stimulatory effects on erythropoiesis.

From the results of this study and others, we suggest that *Panax ginseng* may stimulate the activity of the bone marrow stem cells and that nutritional supplementation with it may help overcome fluorotic anemia in humans as well as in animals. However, further research is required to investigate its effects on the regulation of the immune system and erythrocyte production in bone marrow.

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