STRAWBERRY FRUIT JUICE AMELIORATES FLUORIDE-INDUCED PATHOPHYSIOLOGICAL ALTERATIONS IN PREGNANT MICE

Muhammad A Kanwal,[a] Kausar Raees,[b] Tahir Abbas,[c] Khawaja R Ahmad[a*]

Sargodha, Pakistan

ABSTRACT: We investigated the pathophysiological alterations resulting from the chronic exposure of pregnant mice to fluoride ions (F) and the remedial capacity of strawberry fruit juice (SFJ) The dams were placed in 5 groups (n=10): control (no treatment); F1 and F2 (5 and 10 mg F/L respectively in drinking water on gestation days 6–18); FS1 and FS2 (F exposure as in F1 and F2 respectively+0.2 mL SFJ 12-hrly) and blood samples were collected for examination on gestation day 18. All the RBC parameters (Hb, RBC count, PCV, MCH, MCHC, and MCV) were significantly higher in the control group compared to the other groups and in some of the F+SFJ-treated groups compared to corresponding F-treated groups (Hb in FS2>F2, RBC count in FS2 >F2, PCV in FS1>F1, MCH in FS2>F2, and MCHC in FS1>F1). For the control group, the WBC and lymphocyte counts were significantly lower, compared to the other groups, while the neutrophil count was higher than in F2 and lower than in FS1. The neutrophils were higher in the two F+SFJ-treated groups compared to the corresponding F-treated groups. The bilirubin was increased in F2 compared to the control group. The SGOT, SGPT, ALP, triglycerides, cholesterol, LDL, VLDL, and blood glucose were increased in all the treated groups, compared to the control group, with the values in the F+SFJ-treated groups being significantly less than in the corresponding F-treated groups. The HDL, total protein, albumin, globulin, and albumin/globulin ratio were all decreased in all the treated groups, compared to the control group, with the decreases in the F+SFJ-treated groups being significantly less than in the corresponding F-treated groups. Our results show that the chronic exposure of pregnant mice to 5 or 10 mg F/L can lead to pathophysiological alterations in various blood parameters and that the regular intake of SFJ can ameliorate these alterations.

Keywords: Hematology; Pregnant mice; Serology; Sodium fluoride; Strawberry fruit juice.

INTRODUCTION

Fluoride (F), the ion of the highly reactive element fluorine, is an established environmental toxicant producing a variety of pathology ranging from dental and skeletal fluorosis to endocrine dysfunction and neurotoxicity,[1-3] although some authorities have regarded water fluoridation as a safe and effective preventive measure against tooth decay that reaches all segments of the population.[4] A 2014 report from New Zealand on behalf of the Royal Society of New Zealand and the Office of the Prime Minister's Chief Science Advisor found that extensive analyses of potential adverse effects have not found evidence that the levels of fluoride used in community water fluoridation schemes contribute any increased risk to public health.[4] Fluoride exposure in animals has been reported to cause decreases in the erythrocyte, leucocyte and platelet counts and in the neutrophil ratio along with changes in various hematological parameters (hemoglobin, hematocrit, mean corpuscular volume, mean cell hemoglobin, and mean cell

[a]Department of Zoology, University of Sargodha, Sargodha Pakistan;[b]Principal, Government College For Women, Farooq Colony, Sargodha, Pakistan; [c]Department of Biology, Government College Kot Momin, District Sargodha, Pakistan; *For correspondence: Khawaja Raees Ahmad, Assistant Professor of Zoology, Department of Zoology, University of Sargodha, Sargodha Pakistan; E-mail: kraees@uos.edu.pk; k.r.ahmad@gmail.com;

hemoglobin concentration.[5-9] Significant increases in a number of serum liver function test enzymes (alkaline phosphatase, alanine aminotransferase, and aspartate aminotransferase), *vis-à-vis* the creatinine and uric acid levels, were reported in broiler chicks on continuous feeding for 14–42 days (starting with the day of hatching) on a diet containing 400–1200 mg F/kg body weight.[10] The serum concentration of malondialdehyde, a lipid peroxidation marker, increased while the total proteins, albumin, and enzymatic indicators of oxidative stress (glutathione, superoxide dismutase, glutathione peroxidase, and catalase) were decreased.[10] In laboratory mice, a high F concentration (226 mg/L) in drinking water during pregnancy and early lactation has been reported to cause lipid peroxidation and oxidative stress among the dams and their lactating pups.[11] We recently reported male reproductive toxicity, and alterations in pregnancy and fetal parameters, in laboratory mice receiving a low concentration (5 mg/L) of F in drinking water.[12, 13]

Fresh strawberry *(Fragaria × ananassa)* fruits have been found to lavishly contain a variety of phytochemicals (vitamin C, anthocyanines, flavonoids, polyphenols, ellagic acid, ellagitannins, and quercetins) with proven anti-oxidative, anti-lipid-peroxidative, and antihemolytic activities.[14-16] The present study investigated the hematological and serological changes induced in pregnant mice with chronic exposure to low concentrations (5 and 10 mg/L) of fluoride ions in their drinking water and the ameliorative effects of strawberry fruit juice (SFJ) on the F-induced toxicity.

MATERIALS AND METHODS

Animal groups and treatments: The present study reports on the hematological and serological analyses of the pregnant mice for which the pregnancy and feto-gestational responses have already been reported and thus there is no change in the ambient housing conditions, treatments, or animal groups.[13] The dams were placed in 5 groups (n=10): control (no treatment); F1 and F2 (5 and 10 mg F/L respectively in drinking water on gestation days 6–18); FS1 and FS2 (F exposure as in F1 and F2 respectively+0.2 mL SFJ 12-hrly).

Blood samplings: The dams were euthanized on gestation day (GD) 18 by cervical dislocation to recover blood for hematological and serological study and tissues for the previously reported study of pregnancy and fetal parameters.[13] A blood sample, of approximately 2mL, was obtained from each dam directly from the heart, after abdominal incision, in a 5cc disposable syringe, and immediately stored in an EDTA-coated vacutainer. Immediately after the first sampling, a further fresh blood sample, of approximately 1mL, was obtained from each dam for hematological studies from the uterine and/or hepatic vein.

Hematological and serological studies: The hematological studies were carried out with the help of standard hematocrit tubes for the PCV (Wintrobe hematocrit method); a Sahlis instrument for the hemoglobin (acid hematin method); a hemocytometer for the RBC and WBC counts (hemocytometric method), and Giemsa staining of the blood smears for the differential leukocyte count of

neutrophils and lymphocytes. The following formulae were employed to calculate the MCV, MCH and MCHC.[17]

$$MCV = \frac{Ht \times 10}{TRBC} \quad fL$$

where MCV = mean corpuscular volume or mean cell volume in femtoliters (fL) or 10^{-15}L; Ht = hematocrit or packed cell volume as a volume percentage; TRBC = total red blood cell count/mm^3

$$MCH = \frac{Hb \times 10}{TRBC} \quad pg$$

where Hb = hemoglobin in g/dL; TRBC = total red blood cell count/mm^3 = red blood cell count number $\times 10^6/mm^3$ = red blood cell count number $\times 10^6/\mu L$ = red blood cell count number $\times 10^{12}/L$

$$MCHC = \frac{Hb \times 100}{Hct} \quad g/dL$$

where MCHC = mean cell hemoglobin concentration in g/dL; Hb = hemoglobin in g/dL; Hct = hematocrit or packed cell volume as a volume percentage

The routine serological estimations of the serum were carried on a MICROLAB-300 with the help of standard kits. The data obtained were analyzed statistically on ANOVA and Duncan's multiple range tests using $SPSS_{20}$ software.

RESULTS

A. HEMATOLOGICAL FINDINGS:

A1. Hemoglobin (Hb): Statistical analysis (ANOVA) showed highly significant variations among the groups ($p \leq 0.001$). The *post hoc* analysis indicated a significant variation ($p \leq 0.05$) between any two groups compared with each other except for the F1 and FS1 groups (Table 1).

A2. Red blood cell count (RBC count): An overall highly significant variation ($p \leq 0.001$) among the groups was observed. *Post hoc* analysis showed a significant difference between the control group and both the F1 and F2 groups ($p \leq 0.05$) while the F1 and F2 groups also differed significantly ($p \leq 0.05$) from each other (Table 1).

A3. Packed cell volume (PCV): An overall highly significant difference ($p \leq 0.001$) was observed among the groups. The *post hoc* analysis indicated a significant variation ($p \leq 0.05$) between any two groups (Table 1).

A4. Mean corpuscular hemoglobin (MCH): An overall highly significant ($p \leq 0.001$) difference was observed among the groups. The *post hoc* analysis indicated no significant difference between the F1 and FS1 groups while all the other groups differed significantly ($p \leq 0.05$) from these two and also from each other (Table 1).

A5. Mean corpuscular hemoglobin concentration (MCHC): The analysis of the data indicated a highly significant difference ($p \leq 0.001$) was present between the groups. *Post hoc* comparison between the groups indicated no significant difference between the F2 and FS2 groups while the other 3 groups not only

differed significantly ($p \leq 0.05$) from each other but also from the F2 and FS2 groups (Table 1).

Table 1. Hematological effects of exposure to sodium fluoride exposure and strawberry fruit juice in pregnant mice (Values are mean±SEM)

Parameters	Groups				
	Control	F1	F2	FS1	FS2
Hb (g/dL) †	14.2±0.15^{a}	13.04±0.17^{b}	11.42±0.17^{c}	13.32±0.23^{b}	12.16±0.20^{d}
RBC count (10^6/mm^3) †	8.36±0.25^{a}	7.7±0.20^{b}	6.66±0.11^{c}	7.88±0.20^{b}	7.18±0.15^{b}
PCV (%)†	50±0.30^{a}	44.38±0.38^{b}	41.5±0.76^{c}	45.38±0.33^{d}	42.52±0.56^{e}
MCH (pg) †	16.6±0.47^{a}	14.34±0.17^{b}	13.7±0.36^{c}	14.9±0.43^{b}	14.81±0.23^{d}
MCHC (g/dL) †	26.98±1.22^{a}	25.32±0.96^{b}	24.4±0.49^{c}	26.24±0.75^{d}	24.12±1.00^{c}
MCV (fL)*	58.58±0.58^{a}	53.56±0.41^{bc}	52.92±0.72^{b}	53.92±0.60^{c}	53.6±0.24^{b}
WBC count (10^3/mm^3)*	8.76±0.32^{a}	9.56±0.22^{b}	10.62±0.25^{c}	9.66±0.47^{b}	10.26±0.30^{c}
Neutrophils (%)*	12.32±0.89^{a}	12.1±0.67^{ab}	11.5±0.92^{b}	13.18±0.40^{c}	12.78±0.46^{a}
Lymphocytes (%)†	73.8±3.8^{a}	82.4±0.92^{bc}	86.6±0.92^{d}	81±0.89^{b}	84.4±0.74^{cd}

Comparing the means of the groups for each parameter: *$p \leq 0.05$, †$p \leq 0.001$; ^{abcd} any two groups in a row not sharing a common lowercase alphabet superscript differed significantly from each other at $p \leq 0.05$, (*post hoc* analysis).

A6. Mean corpuscular volume (MCV): Analysis of the data showed a significant variation ($p \leq 0.05$) among the groups. The *post hoc* analysis indicated a significant difference ($p \leq 0.05$) between the control group and the other four groups, while the FS1 group differed significantly with all others except F1 group (Table 1).

A7. White blood cell count (WBC count): The analysis of the total leukocyte count data showed a significant variation ($p \leq 0.05$) was present among the groups. The F- treated groups, F1 and F2, differed significantly ($p \leq 0.05$) from the control group in the *post hoc* analysis. No significant difference was present between the F1 and FS1 groups or between the F2 and FS2 groups (Table 1).

A8. Neutrophils: Analysis of the differential leukocyte count data for neutrophils showed a significant variation ($p \leq 0.05$) was present among the groups. The *post hoc* analysis showed a significant difference ($p \leq 0.05$) was present between the F1 and FS1 groups, and between the F2 and FS2 groups (Table 1).

A9. Lymphocytes: Analysis of the differential leukocyte count data for lymphocytes showed a highly significant variation ($p \leq 0.001$) among the groups.

The *post hoc* analyses indicated significant variations ($p \leq 0.05$) were present between the control group and the other four groups. No significant variation was present between the F1 and FS1 groups or between the F2 and FS2 groups (Table 1).

B. SEROLOGICAL FINDINGS:

B1. Bilirubin: Analysis of the data showed a significant variation among the groups ($p \leq 0.05$). *Post hoc* analysis indicated a significant ($p \leq 0.05$) difference between the control and F2 groups but no difference between F1 and FS1 or between F2 and FS2 (Table 2).

Table 2. Serological effects of exposure to sodium fluoride exposure and strawberry fruit juice in pregnant mice (Values are mean±SEM)

Parameters	Groups				
	Control	F1	F2	FS1	FS2
Bilirubin (mg/dL)*	0.55±0.01^{a}	0.58±0.02^{ab}	0.6±0.02^{b}	0.56±0.01^{a}	0.57±0.02^{ab}
SGOT or AST (U/L)‡	101.7±1.21^{a}	140±1.93^{b}	142.5±4.42^{b}	109.7±1.39^{c}	129.3±2^{d}
SGPT or ALT (U/L)‡	60.25±0.59^{a}	65.91±4.51^{b}	85.91±0.51^{c}	42.75±0.67^{d}	60.4±1.93^{a}
ALP (U/L)‡	161.8±1.54^{a}	201.2±2.03^{b}	251±10.81^{c}	174.2±12.34^{d}	195±6.58^{b}
Total protein (g/dL)‡	7.59±0.06^{a}	6.08±0.04^{b}	5.02±0.04^{c}	7.02±0.14^{d}	6.73±0.05^{e}
Albumin (g/dL)‡	4.2±0.07^{a}	3.22±0.02^{b}	2.5±0.03^{c}	3.66±0.04^{d}	3.61±0.03^{d}
Globulin (g/dL)‡	3.39±0.03^{a}	2.86±0.03^{b}	2.52±0.04^{b}	3.36±0.03^{c}	3.12±0.13^{d}
A/G ratio‡	1.24±0.02^{a}	1.09±0.03^{b}	0.99±0.02^{b}	1.13±0.04^{c}	1.16±0.005^{c}
Triglycerides (mg/dL)‡	80.7±1.65^{a}	159.9±3.04^{b}	181.1±1.01^{c}	124.8±5.64^{d}	131.7±4.27^{e}
Cholesterol (mg/dL)‡	90.45±9.91^{a}	131.3±2.64^{b}	138.2±3.65^{b}	118.8±2.75^{c}	127.1±0.80^{b}
HDL (mg/dL)‡	40.8±0.32^{a}	32±0.47^{b}	25.9±0.45^{c}	35.8±1.008^{d}	35.1±0.37^{d}
LDL (mg/dL)†	39.9±0.82^{a}	55.4±1.89^{b}	56±0.51^{b}	41.2±0.41^{c}	43.5±1.52^{d}
VLDL (mg/dL)†	25.8±1.13^{a}	31.8±1.06^{b}	34.2±0.41^{c}	26.4±1.77^{a}	27.1±0.70^{a}
Blood glucose (mg/dL)‡	111.2±3.60^{a}	155.1±0.65^{b}	159.7±0.49^{b}	113.7±0.68^{a}	148.9±3.81^{c}

Comparing the means of the groups for each parameter: *$p \leq 0.05$, $^{†}p \leq 0.001$; $^{‡}p \leq 0.0001$; ^{abcd} any two groups in a row not sharing a common lowercase alphabet superscript differed significantly from each other at $p \leq 0.05$, (*post hoc* analysis).

B2. Serum glutamate oxaloacetate transaminase (SGOT): The data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis

indicated a significant increase ($p \leq 0.05$) was present in the SGOT levels in the F-treated and the F+SFJ-treated groups compared to the control group. Although it remained significantly higher than in the control group, the mean SGOT level in the FS1 and FS2 groups was significantly less ($p \leq 0.05$) compared to the F1 and F2 groups respectively (Table 2).

B3. Serum glutamic pyruvic transaminase (SGPT): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significantly elevated ($p \leq 0.05$) mean SGPT level in the F1 and F2 groups compared to the control group, with the levels in the FS1 and FS2 groups being significantly less ($p \leq 0.05$) than those in the corresponding F-treated groups, F1 and F2 (Table 2).

B4. Alkaline phosphatase (ALP): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant ($p \leq 0.05$) increase in the mean ALP levels in both the F-treated and the F+SFJ-treated groups compared to the control group. The mean ALP levels in the FS1 and FS2 groups were significantly lower ($p \leq 0.05$) than in the F1 and F2 groups, respectively (Table 2).

B5. Plasma total protein (TP): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis showed a significant decrease ($p \leq 0.05$) in the mean plasma TP in both the F-treated and the F+SFJ-treated groups compared to the control group, with the reductions being significantly less ($p \leq 0.05$) in the F+SFJ-treated groups, FS1 and FS2, compared to the corresponding F-treated groups, F1 and F2 (Table 2).

B6. Plasma albumin (PA): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant decrease ($p \leq 0.05$) in the mean PA levels in both the F-treated and the F+SFJ-treated groups compared to the control group, with the reductions being significantly less ($p \leq 0.05$) in the F+SFJ-treated groups, FS1 and FS2, compared to the corresponding F-treated groups, F1 and F2 (Table 2).

B7. Plasma globulin (PG): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant decrease ($p \leq 0.05$) in the mean PG levels in both the F-treated and the F+SFJ-treated groups compared to the control group, with the reductions being significantly less ($p \leq 0.05$) in the F+SFJ-treated groups, FS1 and FS2, compared to the corresponding F-treated groups, F1 and F2 (Table 2).

B8. Albumin/globulin ratio (A/G ratio): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant decrease ($p \leq 0.05$) in mean the A/G ratio in both the F-treated and the F+SFJ-treated groups, compared to the control group, with the reductions being significantly less ($p \leq 0.05$) in the F+SFJ-treated groups, FS1 and FS2, compared to the corresponding F-treated groups, F1 and F2 (Table 2).

B9. Triglycerides (TG): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant

increase ($p \leq 0.05$) in the mean TG levels in both the F-treated and the F+SFJ-treated groups compared to the control group, with the elevations being significantly less ($p \leq 0.05$) in the F+SFJ-treated groups, FS1 and FS2, compared to the corresponding F-treated groups, F1 and F2 (Table 2).

B10. Cholesterol: Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant increase ($p \leq 0.05$) in the mean cholesterol levels in both the F-treated and the F+SFJ-treated groups compared to the control group, with the elevations being significantly less ($p \leq 0.05$) in the F+SFJ-treated group, FS1, compared to the corresponding F-treated group, F1 (Table 2).

B11. High density lipoproteins (HDL): Analysis of the data showed highly a significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant decrease ($p \leq 0.05$) in mean the HDL levels in both the F-treated and the F+SFJ-treated groups compared to the control group, with the decreases being significantly less ($p \leq 0.05$) in the F+SFJ-treated groups, FS1 and FS2, compared to the corresponding F-treated groups, F1 and F2 (Table 2).

B12. Low density lipoproteins (LDL): Analysis of the data showed a significant variation ($p \leq 0.01$) among the groups. *Post hoc* analysis indicated a significant increase ($p \leq 0.05$) in mean the LDL levels in both the F-treated and the F+SFJ-treated groups compared to the control group, with the increases being significantly less ($p \leq 0.05$) in the F+SFJ-treated groups, FS1 and FS2, compared to the corresponding F-treated groups, F1 and F2 (Table 2).

B13. Very low density lipoproteins (VLDL): Analysis of the data showed a significant variation ($p \leq 0.01$) among the groups. *Post hoc* analysis indicated a significant increase ($p \leq 0.05$) in mean the VLDL levels in the F1 and F2 groups compared to the control group. Compared to the control group, non-significant increases in the mean VLDL levels occurred in the FS1 and FS2 groups and the levels in the FS1 and FS2 groups were significantly lower ($p \leq 0.05$) than in the F1 and F2 groups, respectively (Table 2).

B14. Blood glucose (BG): Analysis of the data showed a highly significant variation ($p \leq 0.001$) among the groups. *Post hoc* analysis indicated a significant increase ($p \leq 0.05$) in the mean BG levels in the F1, F2, and FS2 groups, compared to the control group. The mean BG levels in the FS1 and FS2 groups were significantly lower ($p \leq 0.05$) than in the F1 and F2 groups, respectively (Table 2).

DISCUSSION

Fluoride exposure studies in animals have shown significant changes in hematological blood parameters (hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, packed cell volume, total count of RBCs and WBCs, and the differential leukocyte count of neutrophils and lymphocytes).[10] F exposure has also been found to lower down the serum protein (albumin and globulins) concentrations and balance.[11] Another profound stress indicator of F exposure is lipid per-oxidation that leads to a decline in the serum HDL levels together with a

simultaneous elevation of the LDL, VLDL, triglyceride, and, cholesterol levels.[12] Similarly increased blood glucose concentration is another important sign of the stress induced by F exposure.[18] The serum enzymatic biomarkers of hepatic lesions (SGOT, SGPT, ALT) have also been found to be elevated significantly on exposure of fluoride.[18,19] However, in all above-mentioned studies the exposure concentration of F was kept very high (100 ppm or above) and, moreover, there has not been a previous study reporting the pathophysiological alterations of F exposure in pregnant animals. Our results indicate significant alterations in various hematological (Hb, RBC count, MCH, MCHC, WBC count, lymphocytes, and neutrophils) and serological (bilirubin, SGOT, SGPT, ALP, TP, AL, TG, cholesterol, LDL and BG) parameters of the pregnant mice at very low concentrations (5–10 ppm) of F exposure. The finding in our study of widespread changes in blood and serological parameters in pregnant mice with exposure to low concentrations of fluoride indicates that in the state of pregnancy the sensitivity towards F exposure is markedly increased.

Susheela et a.l[20] recently reported a significant decrease in the hemoglobin in aluminum smelter workers occupationally exposed to fluoride in the air and/or water. In this context, the F exposure-induced thyroid insufficiency (causing a probable decline in erythropoietic stimulation) and a simultaneous destruction of probiotics (leading to diminished synthesis of vitamin B12 which is needed for the biosynthesis of hemoglobin) have been claimed to cause reduced erythropoiesis and the resultant anemia.[21] Similar mechanisms may have been involved in the F-exposed dams in the present study. Increased destruction of RBCs, as indicated by a significant rise in the plasma bilirubin concentration, may also be an additional cause of the significant decrease in the Hb, RBC count, PCV, MCH, and MCHC parameters in the F-exposed dams compared to those in the control group. The noticeable recovery in all these parameters in the SFJ-treated groups, FS1 and FS2, compared to their respective F-treated groups, F1 and F2, indicates the ameliorative effect of SFJ against the F-induced decline in the erythropoietic capacity of the dams. Fluoride exposure, at 100 ppm, in the drinking water has been found to cause a decline in the neutrophils and the WBC count in rats, while the lymphocyte count was noticeably increased.[9] In the present study, we found a similar decline in both the neutrophils and the WBC count, with a simultaneous significant rise in the lymphocytes, in pregnant mice, with a far less F exposure concentration of 5 ppm. Thus, despite the general view that fluoride is systemically toxic, it appears that F may enhance humoral and cell-mediated immunity, as exposure to fluoride leads to an increase in the lymphocytes which are primarily produced in the soft tissues of the lymph nodes and thymus. In contrast, the decrease in the neutrophils, which are primarily produced in the bone marrow, may by attributable to its anti-mitotic effect on the bones, even at low exposure concentrations.[22] The simultaneous exposure to SFJ was found to considerably enhance both the neutrophils and the lymphocytes indicating the general immune promoting effect of SFJ in pregnancy.

In an *in vitro* study 80 µg F/mL exposure was found to cause mitotic arrest and DNA damage in cultured human hepatocytes.[23] Fluoride exposure, injected on a weekly basis in a dose of 10, 20, or 30 µg/g body weight, for four weeks in day-old domestic chicks, caused a significant increase in the serum concentrations of SGOT, SGPT, ALP, and bilirubin indicating its hepatotoxic effect *in vivo*.[24] Similarly, an elevation in lipid peroxidation, and increased glutathione s-transferase, and xanthine oxidase activity, with a simultaneous decrease in catalase and superoxide dismutase activity, were noted in Wistar female mice exposed to 5 and 10 ppm NaF in drinking water for three months.[25] These findings are in agreement with the results of the present study where we noted significant rises in the bilirubin, SGOT, SGPT ALP, cholesterol, TG, LDL, and VLDL levels in both the F1 and F2 groups, compared to the control group, indicating similar hepatotoxic and oxidative stress effects on the pregnant mice. We also found an elevated blood glucose in the F-exposed mice which is yet another sign of stressed gluconeogenesis and a logical consequence of the elevated SGOT and SGPT activities. The results show that co-treatment with strawberry fruit juice has an ameliorative effect on these F-exposure stress effects. There is, therefore, a clear indication that strawberry fruit juice has an exceptional capacity for the normalizing the pathophysiological indicators of F-exposure stress in pregnancy.

CONCLUSION

The findings of the present study show that chronic exposure to a low dose fluoride in drinking water, at 5 and 10 mg F/L, can cause serious alterations in the hematological and serological indicators of metabolic and cytological stress, while the twice-daily consumption of strawberry fruit juice, at 12-hourly intervals, can effectively ameliorate these changes. This clearly illustrates that strawberry fruit juice has health promoting effects, especially for pregnant dams.

ACKNOWLEDGEMENTS

We gratefully acknowledge the administration and relevant bodies of the University of Sargodha, Sargodha, Pakistan, for the granting permission for the study to be done, for providing the laboratory and animal house facilities, and for providing the funds to conduct the research.

REFERENCES

1 National Research Council (NRC). Fluoride in drinking water: a scientific review of EPA's standards. Washington, DC: National Academies Press; 2006.
2 Shivaprakash PK, Ohri K, Noorani H. Relation between dental fluorosis and intelligence quotient in school children of Bagalkot district. J Indian Soc Pedod Prev Dent 201; 29:117-20.
3 Choi AL, Sun G, Zhang Y. Grandjean P. Developmental fluoride neurotoxicity: a systematic review and meta-analysis. Environ Health Perspect 2012; 120:1362-8.
4 Eason C, Elwood JM, Seymour G, Thomson WM, Wilson N, expert panel appointed by the Royal Society of New Zealand; Bardsley A, report preparer; Prendergast KL, panel lay observer; Temple W, New Zealand reviewer; Coggan D, Ferguson MWJ, Reynolds E, international reviewers. Health effects of water fluoridation: a review of the scientific evidence. Auckland and Wellington; Office of the Prime Minister's Chief Science Advisor and Royal Society of New Zealand; 2014, corrected and republished 2015. Available from: http://www.royalsociety.org.nz/media/2014/08/Health-effects-of-water-fluoridation_Aug_2014_corrected_Jan_2015.pdf

5 Karram MH, Ibrahim TA. Effect of industrial fluorosis on haemogram of camels.Fluoride 1992; 25:23-36.
6 Mittal M, Flora SJS. Vitamin E supplementation protects oxidative stress during arsenic and fluoride antagonism in male mice. Drug Chem Toxicol 2007;30:263-81.
7 Arpita S, Bidyut B. Effect of fluoride toxicity on some clinical, biochemical and physiological aspects of albino rats. Int J Res Chem Environ 2012;2: 60-5.
8 Khan AM, Dubey N, Raina R, Singh G, Beigh SA. Toxic effects of deltamethrin and fluoride on hematological parameters in rats. Fluoride 2013;46:34-8.
9 Atmaca N, Yıldırım E, Güner B, Kabakçı R, Bilmen FS. Effect of resveratrol on hematological and biochemical alterations in rats exposed to fluoride. BioMed Res Int 2014;2014:698628. doi: 10.1155/2014/698628. Epub 2014 Jun 5.
10 Deng Y, Cui H, Peng X, Fang J, Zuo Z, Deng J. Luo Q. Effects of high dietary fluoride on serum biochemistry and oxidative stress parameters in broiler chickens. Health 2014;6: 1840-8.
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: 339-49.
12 Ahmad KR, Nauroze T, Raees K, Abbas T, Kanwal MA, Noor S, Jabeen S. Protective role of jambul (*Syzygium cumini*) fruit-pulp extract against fluoride-induced toxicity in mice testis: a histopathological study. Fluoride 2012;45(3 Pt2):281-9.
13 Ahmad KR, Kanwal MA, Raees K, Abbas T. Strawberry fruit extract ameliorates pregnancy and feto-gestational effects of sodium fluoride exposure in mice. Fluoride 2015; 48(1):62-8.
14 Giampieri F, Tulipani S, Alvarez-Suarez JM, Quiles JL, Mezzetti B, Battino M. The strawberry: composition, nutritional quality, and impact on human health. Nutrition 2012; 28: 9-19.
15 Giampieri F, Alvarez-Suarez JM, Mazzoni L, Forbes-Hernandez TY, Gasparrini M, Gonzàlez-Paramàs AM, et al. An anthocyanin-rich strawberry extract protects against oxidative stress damage and improves mitochondrial functionality in human dermal fibroblasts exposed to an oxidizing agent. Food Funct 2014;5: 1939-48.
16 Alvarez-Suarez JM, Giampieri F, Tulipani S, Casoli T, Di Stefano G, González-Paramás AM, et al. One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans. J Nutr Biochem. 2014;25:289-94.
17 Kemal J. Laboratory manual and review on clinical pathology. Foster City, CA, USA: OMICS Group eBooks; 2014. pp. 4-17. Available from: http://esciencecentral.org/ebooks/Laboratory/pdf/laboratory-manual-and-review-on-clinical-pathology.pdf
18 Vasant RA, Narasimhacharya A. Amla as an antihyperglycemic and hepato-renal protective agent in fluoride induced toxicity. J Pharm Bioallied Sci 2012;4:250-4.
19 Michael M, Barot VV, Chinoy NJ. Investigations of soft tissue functions in fluorotic individuals of North Gujarat. Fluoride 1996;29:63-71.
20 Susheela AK, Mondal NK, Singh A. Exposure to fluoride in smelter workers in a primary aluminum industry in India. Int J Occup Environ Med 2013;4:61-72.
21 Hillman D, Bolenbaugh DL, Convey EM. Hypothyroidism and anemia related to fluoride in dairy cattle. J Dairy Sci 1979;62:416-23.
22 Thanga PS, Milton PS. Epigallocatechin gallate potentially ameliorates sodium fluoride-induced DNA damage, and genotoxicity in the bone marrow cells of rats. Asian J Pharmacol Toxicol 2013; 1(2): 8-16.
23 Wang AG, Xia T, Ru R, Yuan J, Chen XM, Yang K, et al. Antagonistic effect of selenium on oxidative stress, DNA damage, and apoptosis induced by fluoride in human hepatocytes. Fluoride 2004;37:107-16.
24 Anjum KM, Mughal MS, Sayyed U, Yaqub A, Khalique A, Rashid MA, et al. Influence of increasing fluoride dose rates on selected liver and kidney enzymes profile in domestic chicken (*Gallus domesticus*). J Anim Plant Sci 2014;24:77-80.
25 Sandeep V, Kavitha N, Praveena M, Sekhar PR, Rao KJ. Alterations of detoxification enzyme levels in different tissues of sodium fluoride (NaF0 treated albino mice. International Journal of Advanced Research 2014;2(1):492-7.