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Fluoride-induced Hematological and Biochemical Changes in Albino Rat. The Therapeutic Action of Vitamin C and Olive Oil on Fluoride Effects

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Abstract: *Hematological and biochemical changes were evaluated in sodium fluoride intoxicants in albino rats. The therapeutic action of vitamin C and olive oil was also determined. Sodium Fluoride administration caused significant decrease in blood parameters such as white blood cells count, platelets count and caused a general decrease in red blood cells count, hemoglobin content, hematocrit value, mean corpuscular volume and mean corpuscular hemoglobin. On the other hand oral administration of sodium fluoride to albino rats, caused a general increase in its biochemical parameters such as urea, uric acid, creatinine, cholesterol and triglycerides; while glucose level decreased significantly. Our results indicate a general decrease of total protein and albumin concentrations, while globulin levels increased. Liver function enzymes such as alnine-amino transferase, aspartate amino transferase and alkaline phosphatase and bilirubin levels were increased as well under the influence of sodium fluoride administration. Signs of improvements in the previous hematological and some biochemical parameters were noticed after the administration of vitamin C or olive oil prior to sodium fluoride administration.*

Keywords: hematological and biochemical parameters – sodium fluoride – vitamin C – olive oil – albino rats.

Introduction

Fluorine is the most electronegative element, distributed ubiquitously as fluorides in nature. Water is the major medium of fluorides intake by humans (WHO, 1984). Fluoride is an element that is not found in free form in nature (Mittal and Flora, 2006). Due to its relatively large electronegativity, fluoride combines with many elements and forms ionized fluorides that are capable of accumulation in the body. Ionized fluorides are reported to be responsible for the clinical symptoms of tissues and organs damage associated

with fluoride intoxication (Chlubek, 2003; Eraslan et al., 2007; Mittal and Flora, 2006; Rzeuski et al., 1998; Shanthakumari et al.1997, and Şireli and Bülbül, 2004). Fluorides may be accumulated in plants or may deposit on its upper parts.The amount of fluoride which can be taken up by plants depend on plant type, soil nature, and the amount of fluoride in soil. Virtually, all food stuff may contain traces of fluoride. All vegetations contain some fluoride, which is absorbed from soil and water. The highest levels in field-grown vegetables are found in curly kale (up to 40 mg/kg fresh weight) and endive (0.3-2.8 mg/kg fresh weight). Other kinds of food contain high levels of fluoride such as fish (0.1-30 mg/kg) and Tea (mg/kg) (EPA, 1985 and Sloof *et al.*, 1988).

In warm areas, dental fluorosis occurs in response to lower concentrations of fluoride in drinking water because a greater amount of water is consumed (WHO, 1984; EPA, 1985 and Cao et al., 1992). Dental fluorosis develops at concentration in drinking water below 1.5 mg/liter (Cao et al., 1992). A decade later, Ginn and Volker reported a nearly 30% reduction in blood hemoglobin in an unspecified strain of rats after 86 days with 50 ppm of fluoride in their drinking water (Ginn and Volker 1944 and Susheela and Jain, 1983).

Hirao (1972) found in rabbits that a 2 to 4 weeks exposure to 10 and 50 mg Sodium fluoride (NaF)/Kg b.wt led to bone marrow hypoplasia, marked by a decreased in total nucleated cell count and anemia. However, other study by Hillmam *et al.*, (1979), disclosed anemia as well as hypothyroidism in dairy cattle as a result of excessive fluoride ingestion. Fluoride, is readily absorbed from the intestinal mucosa, and combines with Ca^{2+} to form calcium ionospheres that easily permeabilize the cell membrane (Sireli and BulBul 2004). The duodenum, proximal jejunum, distal ileum, and colon absorb proportionally to its luminal concentration via passive diffusion (Garzouli and Senator, 1994 and Whitford, 1996). Fluoride can rapidly cross the cell membrane and distributed in skeletal and cardiac muscle, liver, skin and erythrocytes (Carlson *et al.*, 1960 and Jacyszyn & Martin, 1986).

Exposure to high concentrations of sodium fluoride in animals and humans has been shown to cause gastrointestinal damage (Shashi, 1999 and Sondhi et al., 1995). Inhabitants of endemic fluorosis areas are often suffer gastric symptoms including loss of appetite, nausea, anorexia, abdominal pain, flatulence, constipation, and intermittent diarrhea (Susheela et al., 1993). In cases of osteofluorosis, intestinal disorders

have also been reported (Czerwinski and Lonkose, 1977). In mammals, acute fluoride intoxication produces a clinical syndrome characterized by nausea, vomiting, abdominal pain, and paresthesias (Gessner et al., 1994). In the present study, the effects of acute fluoride intoxication on the hematological and biochemical parameters were assessed. The therapeutic action of vitamin C or olive oil was also evaluated against fluoride toxicity in albino rats.

Most acute poisonings in humans caused by fluorides are associated with suicidal or accidental deglutition of fluorine-containing preparations such as insecticides. Most often, poisoning results from the intake of sodium fluoride, sodium fluosilicate, and fluosilicic acid .Fluorides inhibit enzymatic processes, particularly metalloenzymes responsible for important vital processes (Indulski et al.,1989). Acute poisoning by fluoride is most often a result of the intake of significant amounts of toxic fluoride compounds such as HF, NaF, or Na2SiF6. Depending on the type of the fluoride compound and dose, various types of adverse responses may occur (Mamczar et al., 2005). In glycolysis, fluoride ions impede the key enzyme of the path, i.e. pyruvate kinase, which participates directly in ATP formation (Guminska and Sterkowicz, 1976). Combustion of fatty acids is also inhibited since fluoride ion blocks Mg-dependent pyrophosphates (Batenburg and Bergh, 1972). An increase in the biosynthesis of lipids during experimental fluorosis can occur as well (Shashi, 1992 and Machoy – Mokrynska at al., 1994).

Fluoride ion affects not only the fat and carbohydrate balance but also protein equilibrium. Furthermore, as a result of fluoride ion action on the human organism, changes in proteometablism become pronounced (Birkner et al., 2000). Free radicals and lipid peroxidation are known to play an important role in fluorosis (Sun et al., 1994). Fluoride induced hepatotoxicity due to the formation of free radicals and decreased activity of the antioxidant system in hepatocytes of animals and humans (Li and Coa, 1994 and (Karimov et al., 2003). Fluoride exposure also induces histopathological changes in liver involving focal necrosis, infiltration of leucocytes, ultrastructural alteration in hepatocytes and increased apoptosis in animals and humans (Sharma & Chinoy, 2000 and Wang *et al*., 2002).

Materials and Methods

Experimental Animals

Animals used in the current investigation were female rats (100-120 gm) from the breeding unit of the Biological Science department at the Islamic University of Gaza. Animals were housed in well aerated cages and left for one week before experiment. They were kept in plastic cages with wire mesh covers and maintained under the following conditions: temperature (20^{o} C-21^{o} C), relative humidity 40%-60% and a light/dark cycle of 14 and 10 hours). A commercial balanced diet and water were regularly supplied during the experimental period. Animals were divided into four different groups and all groups have the same number of rats (n= 6), the first group is a control group which were forced fed with 1ml of distilled water orally by means of a stomach tube. The second group received a single dose of sodium fluoride (50 mg/kg.bwt) orally, approximately to dose of (Brinker et al., 2000). The third group was given vitamin C (150 mg/kg/bwt dissolved in DW) according to (El-Nahas 1993) orally then, after 2 hours, sodium fluoride (50 mg/kg b.wt) was given orally. The fourth group was given olive oil (1ml/kg.bwt) according to (Abdel Aziz 2000) orally then, after 2 hours, sodium fluoride (50 mg/kg.bwt) was given orally to all treated rats.

After 2 hours all animals were decapitated according to (Birkner et al., 2000) and blood samples were collected in two different sterile test tubes, the first one has Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. These samples were used for hematological examination. The second tube was without any additives which was separated by centrifugation at 3000 r.p.m. for 20 min. Clear serum samples were collected and stored in deep freezer at (-20 ^{o}c) for biochemical analysis.

Biohemical analysis

Serum samples were analyzed for glucose, triglycerides and total cholesterol by the methods described by Trinder (1969), Fossati and Prencipe (1982) and Allain *et al.*, (1974), respectively. Non-protein nitrogen constituents were determined by the methods of Mackay and Mackay (1927) for urea, Fossati *et al*., (1980) for uric acid and Bartels *et al*., (1972) for creatinine. Enzymes activities were measured using

Boehringer reagent kits. The activity of aspartate amino transferase (AST) and alanine aminotransfarse (ALT) were determined as described by Reitman and Frankel, (1957). The activity of alkaline phosphatase (ALP) was measured according to Bessey *et al.,* (1946). Total serum bilrubin, was measured according to Doumus *et al.,* (1937).

Total protein was determined using photometric test according to biuret method (Zaia, *et al*., (1998). Albumin was determined using photometric test using bromocresol green assay (Gustafsson *et al*., (1976).

Hematology analysis

All blood samples collected with anticoagulant were analyzed manually for complete blood count and RBC's indices were calculated.

Statistical analysis

Data are presented as means ± SD of triplicate observations. Two sample T-test and confidence interval (CI) analysis ($P \leq 0.05$ = 95% CI) was used to compare mean values using SPSS program.

Results

The effect of water in the first group of albino rats was initially tested and its results were used as a control to compare with the other groups. In the second group, sodium fluoride administration results in a significant change in the hematological and biochemical parameters. A high significant decrease ($p<0.05$) in the total WBC's count with a recorded difference of 48.24% as compared with the control group (the first group). However, a significant decrease in RBC's count with a recorded percentage change 11.18%, a decrease in HB measurements with a recorded percentage change 14.79% and a non-significant decrease in most RBC's indices as shown in Table 1.

The administration of vitamin C and olive oil showed a significant reduction of sodium fluoride effects which reversely affect the albino rat hematological parameters especially the total WBC's count with a recorded difference of 24.4 and 12.10 respectively as compared with 48.24% difference caused by the administration of sodium fluoride alone in the second group. Reduction effect was also noticed on the other hematological parameters such as RBC count with a recorded

percentage change of 5.98 and 5.01 compared with 11.18% when sodium fluoride administered alone. Hemoglobin (HB) measurements were improved with the administration of vitamin C and olive oile with a recorded percentage change of 9.35 and 7.18 respectively as compared with 14.79% difference caused by the administration of sodium fluoride alone and caused a general decrease in most RBC's indices as shown in Table 1.

Table (1): The effect of the oral administration of sodium fluoride on albino rats blood indices with /without the treatment of vitamin C or olive oil.

Parameters	Control n=6	NaF n=6	NaF + Vit.C n=6	NaF + Olive oil n=6
WBC's count (*10^3 cell/ul)	5.12 ± 0.55	2.65 ± 0.35	3.85 ± 0.59	4.50 ± 0.75
%change		-48.24	-24.80	-12.10
P value		< 0.01	< 0.01	< 0.05
RBC's count (x10^6 cell/ul) %change	6.89 ± 0.14	6.12 ± 0.15	6.54 ± 0.22	6.48 ± 0.25
P value		-11.18	-5.07	-5.95
		< 0.05	> 0.05	> 0.05
Hb (g/dl) %change	13.79 ± 0.25	11.75 ± 0.30	12.50 ± 0.16	12.80 ± 0.17
P value		-14.79	-9.35	-7.18
		< 0.05	> 0.05	> 0.05
HCT (%)	39.60 ± 0.30	33.75 ± 0.36	36.90 ± 0.19	37.0 ± 0.31
%change		-14.77	-6.81	-6.56
P value		< 0.05	> 0.05	> 0.05
MCV (f1)	56.73 ± 1.12	55.18 ± 0.91	56.42 ± 0.66	57.10 ± 0.76
%change		-2.79	-0.54	0.65
P value		> 0.05	> 0.05	> 0.05
MCH (pg)	19.76 ± 0.24	19.20 ± 0.18	19.11 ± 0.26	19.75 ± 0.33

%change		-2.83	-3.28	-0.05
P value		> 0.05	> 0.05	> 0.05
MCHC (g/dl)	34.82 ± 0.60	34.81 ± 0.41	33.88 ± 0.36	43.60 ± 0.39
%change		-0.02	-2.69	-0.63
P value		> 0.05	> 0.05	> 0.05
PLT ($x10^3/41$)	640.95 ±	881.0 ± 16.30	685.50 ±	727.50 ±
%change	10.6	37.45	17.24	18.33
P value		< 0.01	6.95	13.50
			> 0.05	< 0.05

***All values are expressed as (Mean +/- SD) (Triplicate readings)**
***Non-significant P>0.05, significant P<0.05, highly significant P<0.01.**

Table (2): The effect of oral administration of sodium fluoride on albino rats biochemical parameters with /without the treatment of vitamin C or olive oil.

Parameters	Control n=6	Sodium Fluoride n=6	Sodium Fluoride + vit.C, n=6	Sodium Fluoride + olive oil, n=6
Glucose (mg/dl)	98.30 ±	70.0 ± 2.60	81.5 ± 2.17	85.8 ± 3.16
%change	2.91	-28.78	-17.09	-12.71
P value		< 0.01	< 0.05	< 0.05
Triglycerides(mg/dl)	138.57	145 ± 2.17	139 ± 3.15	135.5 ± 2.16
%change	± 251	4.64	0.31	-2.21
P value		> 0.05	> 0.05	> 0.05
Cholesterol(mg/dl)	229.16 ±	235.5 ± 2.10	230 ± 3.19	225.5 ± 2.19
%change	2.63	2.76	0.36	-1.59
P value		> 0.05	> 0.05	> 0.05
Urea(mg/dl)	29.11 ±	32.9 ± 1350	32 ± 1.47	32.5 ± 1.38
% change	1.46	13.01	9.92	11.64
P value		< 0.05	> 0.05	< 0.05

Uric acid (mg/dl) % change P value	4.10 ± 1.12	4.95 ± 1.20 20.7 < 0.01	4.65 ± 1.35 13.41 < 0.05	4.75 ± 1.27 15.85 < 0.05
Creatinine(mg/dl) %change P value	0.90 ± 0.03	1.21 ± 0.02 34.44 < 0.01	1.12 ± 0.03 24.44 < 0.01	1.15 ± 0.04 27.77 < 0.01
Total Protein (mg/dl) %change P value	7.88 ± 0.15	7.30 ± 0.14 -7.36 > 0.05	7.74 ± 0.13 -1.77 > 0.05	7.60 ± 0.15 -3.55 > 0.05
Albumin (mg/dl) %change P value	3.92 ± 0.11	3.0 ± 0.12 -23.46 < 0.01	3.70 ± 0.10 -5.61 > 0.05	3.45 ± 0.13 -11.9 < 0.05
Globulin(mg/dl) %change P value	3.96 ± 0.18	4.30 ± 0.17 8.58 > 0.05	4.04 ± 0.12 2.02 > 0.05	4.15 ± 0.11 4.7 > 0.05
AST (u /m1) % change P value	33.30 ± 2. 20	39.5 ± 1.77 18.61 < 0.05	37.10 ±1.80 11.41 < 0.05	38.20 ± 1.17 14.7 < 0.05
ALT (u /m1) % change P value	39.19 ± 1.45	42.5 ± 1.51 8.44 > 0.05	42.0 ± 1.38 7.17 > 0.05	41.5 ± 1.44 5.89 > 0.05
Al.phosphatase(1u/m1) %change P value	40.16 ± 1.90	43.5 ± 1.80 8.31 > 0.05	41.5 ± 1.15 3.33 > 0.05	42.10 ± 1.50 4.83 > 0.05
Bilirubin total (mg/dl) %change P value	0.75 ± 0.02	0.95 ± 0.01 26.66 < 0.01	0.80 ± 0.02 6.66 > 0.05	0.90 ± 0.03 20 < 0.01

***All values are expressed as (Mean +/- SD) (Triplicate readings)**

***Non-significant P>0.05, significant P<0.05, highly significant P<0.01.**

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) was decreased in response to sodium fluoride administration recording 4.35% and 4.40%, respectively as compared to control level. On other hand, Sodium fluoride administration and treatment with vitamin C resulted in a decrease by 0.54% and 3.28% respectively, compared to control level (Table1). Sodium fluoride administration generally caused an insignificant decrease in the mean corpuscular hemoglobin concentration (MCHC) with a value of 0.02% of the control level. On the other hand, sodium fluoride administration caused a significant increase in blood platelet (PLT) count with a value of 37.45% compared to the control level. However, sodium fluoride administration and treatment with vitamin C or olive oil caused an increase by 6.95% and 13.50% respectively compared with control level.

The mean values of serum total protein, albumin and globulin of both control and experimental animals are presented in (table 2). Total protein concentration in rat blood serum treated with fluoride was decreased by 7.36%, while in rats treated with sodium fluoride in addition to vitamin C or olive oil was 1.77% and 3.55% respectively in comparison with control (table 2). Albumin content in rat serum under the influence of sodium fluoride decreased by 23.46%, while in rats treated with sodium fluoride in addition to vitamin C or olive oil was 5.61% and 11.9% respectively in comparison with control level. Globulin concentration in rats treated with sodium fluoride increased by 8.58%, while in rats treated with sodium fluoride plus vitamin C or olive oil the increase was 2.02% and 4.7% respectively in comparison with the control level.

Under the influence of sodium fluoride the activity of ALT was increased in rat's blood by 8.44%. While in rats treated with sodium fluoride and vitamin C or olive oil, the increase of ALT enzyme activity was 7.17% and 5.89% respectively in comparison with the control (Table 3). The activity of AST was also increased significantly in rat's blood by 18.61% in response to sodium fluoride compared with control (Table 3). However, fluoride administration in addition to vitamin C or olive oil lowers the AST activity to 11.41% and 14.7% respectively, compared with control level.

Alkaline phosphatase activity and bilirubin in rat's blood increased by 8.31% and 26.66% respectively compared to control level after

sodium fluoride administration (table 3), this increase caused by NaF administration was non-significant ($P>0.05$). However, in bilirubin, the increase was significant ($P<0.05$) in the intoxicated rat's and the treated rat's with olive oil, while vitamin C had amilodrate the effect of NaF intoxication ($P>0.05$). The data in table (2) showed that sodium fluoride administration caused a general decrease in serum glucose at the end of experiment. Glucose decrease to 17.09% compared with the control level, after sodium fluoride administration. However, vitamin C failed to raise serum glucose levels in sodium fluoride intoxicated rats to the control level. While olive oil treatment was more effective in reversing sodium fluoride intoxication action on serum glucose.

Table (2) revealed that rat serum triglycerides increased in response to treatment with sodium fluoride. On the other hand, vitamin C positively affected serum triglycerides level, hence it reached, more or less, nearly the level of healthy rats. However, administration of olive oil was less effective to overcome sodium fluoride induced increment in serum triglycerides content in fluoride intoxicated rats compared to the control. Sodium fluoride induced a general elevation in the concentration of cholesterol in intoxicated rat serum in comparison with the control level (table 2). On the other hand, treatment of intoxicated rats with vitamin C lowered the concentration of serum cholesterol towards the control level. Administration of olive oil failed to reverse fluoride induced increment in serum cholesterol level in intoxicated rats. Non-protein nitrogen constituents of rat serum i.e. urea, uric acid and creatinine were affected by the administration of sodium fluoride with/without vitamin C or olive oil. The results were cited in table (2).

Sodium fluoride administration caused a significant increase in rat serum urea with a value of 13.01% compared to the control level. However, sodium fluoride plus vitamin C or olive oil increased serum urea level by 9.92% and 11.64% respectively compared with control level. In general, uric acid and creatinine content in rat blood serum increased in response to the oral administration of sodium fluoride with values of 20.7% and 34.44% respectively compared to the control level (table 2). However, in sodium fluoride plus vitamin C treatment, uric acid and creatinine were increased by 13.41% and 24.44% respectively compared with control level. On other hand, sodium

fluoride administration and treatment with olive oil elevated uric acid and creatinine in rat serum by -15.85%, and 27.77 respectively compared with control level.

Discussion

It is now well established that fluoride ingestion not only affects the teeth and bones but also other organs (Aydin *et al.,* 2003). Chronic administration of fluoride can severely damage many systems of the human body (Ya-Nan *et al.*, 2000) and induces various changes in some organs of animals (Waldbott *et al.*, 1978; Mullenix *et al.*, 1995). Although an association between environmental exposure to fluoride and morbidity from hematological disease has been reported (Machoy, 1990). Toxic effects of fluoride on various body organs have received considerable attention (Spittle and Durgstahler, 1998 and Spittle 2000). The potential influence of fluoride on hematopiesis remains unclear. Fluoride can also have more serious effects on skeletal tissues and cause skeletal fluorosis (with adverse changes in bone structure) which is observed when drinking water contains 3-6 mg of fluoride per liter. Our study demonstrates a link between fluoride administration and blood parameters in albino rats. The treatment of albino rats with sodium fluoride alone exhibited a significant decrease in WBC's count of albino rats. These findings are supported by Susheela and Jain (1983) who found that leukocytes count was significantly reduced after 6-12 months of fluoride ingestion in rabbits. However, most of these changes were improved when vitamin C or olive oil were administered before sodium fluoride compared to sodium fluoride alone. On the other hand, general declines were recorded RBCs, Hb, Ht, MCV, MCH and MCHC but PLT was increased versus the control group. These findings may be due to the inhibitory action of fluoride on the haemopietic system and / or due to the destructive effect of fluoride on viable RBCs. The results of the present investigation are also in agreement with the findings given by Susheela and Jain (1983) who conclude that excessive fluoride ingestion exerts a toxic influence on the hematological profile of adult rabbits. The spleen plays a significant hematopoietic role particularly during the fetal life (Sty & Conway, 1985 and Sieff & Williams, 1995).

Toxic damage to the spleen caused by sodium fluoride may therefore have marked negative effects on hematopoiesis (Anna *et al*., 2002). Oral administration of sodium fluoride caused marked

hypoglycemia in treated rats. Bdrot (1998) reported a significant decline in blood glucose levels in a fluoride affected human population of North Gujarat, India. However, hyperglycemia was reported in rats treated with fluoride (Rigalli *et al*., 1990, and Sakura *et al*., 1993) and in exposed to environmental fluoride from aluminum smelters (Kendryal *et al*., 1993). These differences might be due to variations in dose, duration of exposure, sensitivity of species and physiological status of the animals. Concerning lipid metabolism, results demonstrated that triglycerides and cholesterol levels were increased in response to sodium fluoride administration to albino rats. The possible explanation of the observed increased in serum triglycerides and cholesterol levels may be due to direct or indirect action of sodium fluoride on lipid metabolism (Baha Oral and Özbaar, 2003).

Determination of non protein nitrogen constituents, urea, uric acid and creatinine levels can indicate the functional state of the kidney. Oral administration of sodium fluoride was found to increase serum urea, uric acid and creatinine in treated albino rats compared with the control animals. Elevation of urea in serum could result (rather) from a defective excretory function of the kidneys, especially since increased urea concentration in blood serum is as a general rule caused by renal insufficiency, with or without obstruction of urinary tracts (Birkner *et al*., 2000). Similar findings are reported by Appleton (1995) who administrated 0.4 m/ml of sodium fluoride /kg of body mass to rats as an intraperitoneal injection and foundation increase in urea concentration. He explained the high serum urea concentration caused by lesions of the kidneys.

Likewise, it can be assumed that the NaF dose caused kidney lesions in tested rats, since high concentration of fluoride in plasma amounting to over 90 u mol/L lead to kidney lesions (Gumonska *et al*., 1990). Oral administration of sodium fluoride showed a general decrease in serum total protein and albumin levels in treated albino rats compared with the control animals. The observed decrease in total serum protein is probably concordant with some disorders related with building new proteins by the liver or may be due to the disruption of hepatic cells (Kishor *et al*., 2008).

The decrease in albumin level for rats treated with sodium fluoride

may be due to liver massive necrosis, deterioration of liver function (Guler *et al*.,1994). Never the less, hypoalbuminemie is a common problem in animals treated with sodium fluoride and is generally attributed to the presence of nephropathy (Porte and Halter, 1981). In view of the consideration that the liver is the site of albumin production the formation or derangement synthesis of albumin could be due to liver damage. Fluoride is well known to affect protein synthesis by causing impairment in polypeptide chain initiation (Macuch *et al*., 1963, Rasmussen, 1982); weak incorporation of amino acids into proteins (Helgeland, 1976), and abnormal accumulation or inhibition of RNA synthesis (Halland, 1979). Decreased protein synthesis during fluorides has been reported (by) (Shashi *et al*., 1987; Chiny *et al*., 1993 and Matk and Mathewes *et al*., 1996). Zang *et al*., (1996) reported a significant decrease in serum protein in individuals with poor nutrition and living in high fluoride areas.

The changes observed by Birkner *et al*., (2000) provide evidence for disruption of protein metabolism in rats exposed to an acute dose of NaF. Tyrtyshik (1992) also found disturbances in protein metabolism in rat liver during experimental anemia induced by NaF. Serum ALT and AST, well-Known markers of liver function were significantly elevated in the fluorotic children, indicating liver cell damage and distributed liver function. Similar results have been reported in earlier studies on fluorotic individuals (Chinoy *et al*., 1992 and Mathews *et al*., 1996) and experimental animals (Tsunoda et al., 1985 and Chinoy, 1991). A slight increase in serum alkaline phosphatase levels was observed in our study, which is consistent with the observations of Teotia *et al*., 1971 & Teotia, 1988. The recovery on co-treatment with vitamin C or olive oil could be attributed to the action of vitamin C or olive oil as free radical scavengers. Wilde and Dya (1998) opined that the toxicity of free radicals is greater if fluoride can impair the production of free radical scavengers such as ascorbic acid and glutathione and this can be prevented by the additional supplementation with vitamin C and E. The antidotal effect of vitamin E acts by preventing the oxidative damage caused by fluoride, which increases peroxides and free radicals of reactive oxygen species in tissues.

Studies by Gupta *et al*., (1996) also revealed that the treatment of vitamin C showed significant improvement in skeletal, clinical and

biochemical parameters in children consuming water containing 4.5 ppm of fluoride. Shusheela (1999) reported that consumption of food rich in vitamin C and E, which act as antioxidants scavenging the free radicals and eliminating them, also reduces the fluoride levels in the body. Finally Vermna and Sherlin (2002) pointed out sodium fluoride induced hypoproteinemia and hypoglycemia in parental and F1-generation rats. These changes were ameliorated by vitamin C. Vitamin C is a water soluble antioxidant which can directly scavenge super oxide and hydroxyl radicals. Many clinical trials have focused on single antioxidants (Pacht *et al*., 1986) and Schectman *et al*., 1991. The action of vitamin C may explain the improved hematological and biochemical parameters in sodium fluoride treated rats.

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