Effect of High Concentration of Sodium Fluoride on Serum Lipid Profile of Male Rabbits: Hypolipidemic Effect of Grape Seed Oil

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Abstract

The present study was undertaken to find out the beneficial effect of natural antioxidant (Grape seed oil) against deleterious effect of water fluoridation (sodium fluoride in drinking water) on serum lipid profile of adult male rabbits. Thirty adult male rabbits were randomly divided into three groups (10 rabbit/ group) and were treated daily for 60 days as follows: 1. Group C: Rabbits of this group were allowed to ad libitum supply of drinking water (control group).2. Group T1: Rabbits of this group were allowed to ad libitum supply of drinking water containing 100 ppm of sodium fluoride (NaF) 3. Group T2: Rabbits of this group were allowed to *ad libitum* supply of drinking water containing 100 ppm sodium fluoride and received 0.35 ml /Kg B.W of grape seed oil(GSO) daily. Fasting blood (for 8-12 hrs) samples were collected at different times 0, 30 and 60 days of the experiment .Blood was drawn by cardiac puncture technique for measuring the following parameters: Lipid profile including: serum triacylglycerol (TAG), Total cholesterol - (TC), High density Lipoprotein - cholesterol (HDL-C), Low density lipoprotein - cholesterol (LDL-C) and very low density lipoprotein cholesterol - (VLDL-C) concentrations. The results revealed that animals exposed to 100 ppm of NaF in drinking water for 60 days showed a case of dyslipidemia manifested by significant increase in serum TC, TAG, LDL-C, and VLDL-C concentrations with a significant decrease in serum HDL-C concentrations. Oral intubation of Grape seed oil concurrently with NaF caused a significant correction of the previous studied parameters manifested by a significant elevation in serum HDL-C , in addition to a significant decrease in serums TC, TAG, LDL-C, and VLDL-C concentrations, In conclusion, the results of this study documented the hypolipidemic effect of GSO against dyslipidemia induced by NaF in adult male rabbits.

KEYWORDS: sodium fluoride, GSO, HDL-C, TAG, Male rabbits, LDL-C, VLDL-C.

Introduction

Sodium fluoride is an inorganic chemical compound with the formula NaF , it is a source of the fluoride ion in diverse applications. It is an ionic compound dissolving to give separated Na^+ and F^- ions .Fluoride anions are widely distributed in the environment in different forms and their compounds are extensively used (ATSDR, 2003).The fluorosis of human beings is mainly caused by drinking water, food, beverages containing fluoride (Buzalaf *et al.*, 2004), tooth paste, mouth rinses, and other dental products (Wang *et al.*, 2000 and Rodrigues *et al.*, 2009); drugs and fluoride dust and fumes from industries using fluoride containing salt and hydrofluoric acid (NRC.,2006 and Ananian *et al.*, 2006).While, that of animals is mainly by drinking water and supplementing feed additives such as calcium monohydrogen phosphate containing high levels of fluoride (Liu *et al.*, 2003).Most food have fluoride concentration ,the highest level is present in shell fish followed by cooked veal ,wheat cereals ,rice and walnuts(PMRA.,2006).

Fluoride intoxication is a serious public health problem in many parts of the world as a result of high fluoride content in ground water and airborne fluoride released from burning of fluoride – load coal. It is now well established that fluoride ingestion not only affects the teeth and bones but also other organs (Xiong *et al*., 2007 and Zhang *et al*., 2007). Intake of high levels of fluoride is known to cause structural changes, altered activities of enzymes, metabolic lesions in the brain and influence the metabolism of lipids (Shivarajashankara *et al.,* 2002).

Chronic flourosis is a slow and progressive process causing symptoms related to several systems, particularly musculoskeletal (Srikanth *et al.,* 2008). Metabolic, functional, and structural damages caused by chronic fluorosis have been reported in many tissues, including myocardium (Cicek *et al.,* 2005), kidney (Nabavi *et al.,* 2013; Inkielewicz and Czarnowski, 2008), liver (Hassan and Yousef, 2009) and brain (Basha *et al.*,2011). However, the toxicity kinetics and pathogenesis of fluoride on whole body still remain unclear. Free radical generation is known to be one of most important mechanism of fluoride toxicity (Nabavi *et al*.,2012 and Yamaguti *et al*.,2013). Chronic fluorosis may enhance lipid peroxidation (LPO) by directly interacting with the cellular membrane and ROS and lowering total antioxidant capacity (Stepniak and Czarnowski, 2010 and Rupal et al., 2011 a).

One product produced from grape pomace waste is grape seed oil, which has the following nutritional properties: cholesterol free, low in saturated fats, contains high-density lipoprotein, and rich in Vitamin E and antioxidants (Arvanitoyannis *et al*., 2006 and Crews *et al.,* 2006), has been used in treatment of free radical producing diseases. It was mentioned that grape seeds derived their medicinal effect from compounds called proanthocyanidin (PCOs), which are generally extracted from grape seeds and black currant and added to a commercial preparation. Grapes proanthocyanidin showed potent antioxidant effects that significantly inhibit lipid peroxidation of polyunsaturated fatty acid in animals and in *vitro* studies (Cheng *et al.*,2007 and Weber *et al.,* 2007) .This study was designed to investigate the beneficial effect of natural antioxidant against high concentration of sodium fluoride in water.

Materials and Methods

Thirty adult male rabbits were randomly divided into three equal groups and were treated daily for 60 days as follows:1. Group C: Rabbits of this group were allowed to *ad libitum* supply of drinking water (control group). 2. Group T1: Rabbits of this group were allowed to *ad libitum* supply of drinking water containing 100 ppm of NaF .3. Group T2: Rabbits of this group were allowed to *ad libitum* supply of drinking water containing 100 ppm NaF and received 0.35 ml /Kg. B.W of GSO (Jellin *et al.,* 2003).

Fasting blood (for 8-12 hrs) samples were collected (by cardiac puncture technique) at different interval 0, 30 and 60 days of the experiment ,centrifuged at 2500rpm for 15 minutes, and then serum samples were liquated and frozen at -20 °C until analysis of serum lipid profile including : triacylglycerol (TAG), Total cholesterol (TC), High density Lipoprotein - cholesterol (HDL-C) were measured using enzymatic kits (Linear chemicals ,Barcelona /Spain) ; Low density lipoprotein - cholesterol (LDL-C) and very low density lipoprotein cholesterol - (VLDL-C) concentrations according to (Friedewald,1972) .Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). Specific group means differences were determined using least significant differences (LSD) as described by (Snedecor and Cochran, 1973).

Results and Discussion

Total serum cholesterol (TC) Concentration.

Tables(1,2,3,4) illustrate that serum TC ,TAG,LDL-C and VLDL-C concentrations are close in all groups (P>0.05) in the pretreated period. After thirty days of treatment, a significant (P<0.05) elevation in concentration of previous parameters was observed in NaF, as compared to NaF plus GSO.Oral intubation of GSO concurrently with NaF to male rabbits caused a significant (P<0.05) depression in the elevated TC,TAG ,LDL-C and VLDL-C concentrations after 30 and 60 days of the experiment compared to T1(NaF) treated group. Further significant elevation (P<0.05) in these parameters were observed at the end of experiment in NaF treated group comparing to control and T2 treated groups.

Table (1): Effect of oral administration of grape seed oil (GSO) on Total serum cholesterol concentration (mg/dl) of adult male rabbits treated with Sodium Fluoride in drinking water for 60 days.

Groups	C	T1	T2
Zero	116.4±2.92 A a	119±3.22 A a	**116.6±2.22** **A a**
30	117.8±1.15 C a	174.8±5.66 A b	**145.4±2.11** **B b**
60	118.4±0.67 C a	247±3.14 A c	**154.4±4.98** **B b**

- Values are expressed as mean ± SE, n = 5 each group. C: control group.
- T1: Animals received 100 ppm NaF in drinking water.
- T2: Animals received 100 ppm NaF in drinking water and 0.35 ml\K.g B.w of GSO.
-Capital letters denote differences between groups, P<0.05 vs. control.
- Small letters denote differences within group(periods).

Table (2): Effect of oral administration of grape seed oil (GSO) on serum Triacylglycerol (TAG) concentration (mg/dl) of adult male rabbits treated with Sodium Fluoride in drinking water for 60 days.

Groups	C	T1	T2
Zero	102.8±3.78 A a	102.2±4.32 A a	**104.6±3.72** **A a**
30	98.2±1.59 B a	24.6±10.83 A b	**103.6±3.94** **B a**
60	102.4±4.08 C a	145±8.27 A c	**118.2±6.58** **B a**

- Values are expressed as mean ± SE, n = 5 each group. C: control group.
- T1: Animals received 100 ppm NaF in drinking water.
- T2: Animals received 100 ppm NaF in drinking water and 0.35 ml\K.g B.w of GSO.
- Capital letters denote differences between groups, P<0.05 vs. control.
- Small letters denote differences within group(periods).

Table (3): Effect of oral administration of grape seed oil (GSO) on serum Low density lipoprotein – cholesterol (LDL-c) concentration (mg/dl) of adult male rabbits treated with Sodium Fluoride in drinking water for 60 days.

Groups	C	T1	T2
Zero	69.64±3.63 A a	70.76±2.66 A a	**68.4±2.52** **A a**
30	70.22±1.65 C a	126.68±3.74 A b	**99.48±2.67** **B b**
60	72.84±1.49 C a	199.08±3.37 A c	**105.48±5.71** **B b**

- Values are expressed as mean ± SE, n = 5 each group. C: control group.
- T1: Animals received 100 ppm NaF in drinking water.
- T2: Animals received 100 ppm NaF in drinking water and 0.35 ml\K.g B.w of GSO.
- Capital letters denote differences between groups, P<0.05 vs. control.
- Small letters denote differences within group(periods).

Table (4): Effect of oral administration of grape seed oil (GSO) on serum Very low density lipoprotein – cholesterol (VLDL-c) concentration (mg/dl) of adult male rabbits treated with Sodium Fluoride in drinking water for 60 days.

Groups	C	T1	T2
Zero	20.56±0.75 A a	20.44±0.86 A a	**20.92±0.74** **A a**
30	19.64±0.31 B a	24.92±2.16 A b	**20.72±0.78** **B a**
60	20.48±0.81 C a	29.0±1.65 A c	**23.64±1.31** **B a**

- Values are expressed as mean ± SE, n = 5 each group. C: control group.
- T1: Animals received 100 ppm NaF in drinking water.
- T2: Animals received 100 ppm NaF in drinking water and 0.35 ml\K.g B.w of GSO.
- Capital letters denote differences between groups, P<0.05 vs. control.
- Small letters denote differences within group(periods).

Depending on the results clarified in Table-5, there were no differences in the mean values of HDL-C in zero time in all experimental groups when compared to each others. A significant (P<0.05)decrease in serum HDL-C was found in group T1 (23.2±0.55) and T2 as compared the control group (27.94±1.51) after 30 days of the experiment. Further significant (P<0.05) decrease was observed after 60 day of NaF treatment in T1 group (19.52±0.35) as compared to T2 (25.28±0.37) and the control (25.08±1.37) groups. Oral intubation of GSO in combination with NaF (T2) group caused significant elevations (P<0.05) in serum HDL-C concentration after 60 days of treatment (25.28±0.37) comparing to NaF treated group and the values seemed to be normalized that of the control

(25.08±1.37). Within the time, significant (P<0.05)differences were observed in NaF treated group T1as comparing to the pretreated period, where there iwas a significant decrease in serum HDL-c concentration in T1 treated group after 30 and 60 days of experiment comparing to the pretreatment period.

Table (5): Effect of oral administration of grape seed oil (GSO) on serum High density lipoprotein – cholesterol (HDL-c) concentration (mg/dl) of adult male rabbits treated with Sodium Fluoride in drinking water for 60 days.

Groups	C	T1	T2
Zero	27.2±1.56 A a	27.8±0.49 A a	**27.28±0.74** **A a**
30	27.94±1.51 A a	23.2±0.55 B b	**25.2±0.41** **B a**
60	25.08±1.37 A a	19.52±0.35 B c	**25.28±0.37** **A a**

- Values are expressed as mean ± SE, n = 5 each group. C: control group.
- T1: Animals received 100 ppm NaF in drinking water.
- T2: Animals received 100 ppm NaF in drinking water and 0.35 ml\K.g B.w of GSO.
- Capital letters denote differences between groups, P<0.05 vs. control.
- Small letters denote differences within group(periods).

Chronic fluoride intake has been recorded to cause hyperlipidemia and oxidative stress by many investigators (Barbier *et al*., 2010 ; Rupal *et al*., 2010 and Rupal *et al*., 2011b) . Rupal and Narasimhacharya(2012) reported significant high level of total lipid,TC,LDL-C and VLDL-C after exposure of rats to 100ppm of NaF for four weeks.Fluoride was found to have an inhibitory effect on hepatic cholesterol and free fatty acid synthesis in fluoride treated rabbits (Shashi, 2003) .

The observed abnormalities in lipoprotein profile after exposure to NaF might be due to over-production of VLDL by the liver or to the decrease in removal of VLDL and LDL from the circulation (Tsutsumi *et al.*, 1995). It could be suggested that the abnormal activities of lipases enzymes seemed to be one of the chief and responsible factors for the rise in serum triglycerides and cholesterol. It appeared that enzymes inhibited by fluoride, such as triglyceride lipase, unspecific esterase and pyrophosphates (Czerny *et al.*, 2000) lead to weaken lipid metabolism and a case of dyslipidemia. Moreover oxidative stress induced by NaF (Bergandi *et al.*, 2010) could be claimed. NaF intoxication registed an increased in lipid peroxidation and loss of membrane integrity which might be important in altered lipid metabolism and closely associated with observed hyperlipidemia (Abd-Alwahab .,2013).Besides,fluoride was found to cause hypercholesterolemia due to lower insulin level(Garcia-Montalvo *et al*.,2009).

According to Nash(2004), up to 45 g of grape seed oil per day raised HDL-Cholesterol levels by 13% and reduced LDL-cholesterol levels by 7% in three weeks (Arvanitoyannis *et al.*, 2006). Following consecutive 12 weeks oral administration of tablets containing 0, 200or 400 mg grape seed extract (calculated as proanthocyandin) to 61 healthy subjects, there was a significant decrease in LDL-C and MDA concentration (Sano *et al.*, 2007).

Also, grape seed treatment corrected dyslipidemia and down-regulated some of lipogenic genes (Del Bas *et al.*, 2009) up-regulated by a high fat diet (Quesada *et al.*, 2009). Several active ingredients in grapes (seed, skin, concentrated juice and grape seed oil) may be attributed to its hypolipidemic effect of the plant phytochemical such as catechin, epicatechinn is the known hypolipidemic active ingredients of the GSO (Yilmaz and Toledo, 2004), in addition to its vitamin C content with well known antioxidant activity (Roh and Kim, 2006). The observation of Del Bas *et al.*, (2005) suggested that grape polyphenols, when consumed during meals, can reduce the amount of circulating proatherogenic lipoproteins by decreasing their production in intestine and liver, while increasing their clearance by the liver. Antioxidant compound present in grape (OPCs) might have the ability to directly scavenge OH* protect LDL from oxidation, reduce serum cholesterol level, and improve lipoprotein profile through lowering the LDL-C concentration(Lun-Yi *et al.*, 2000 and Pinelo *et al.*,2006),as well as elevated HDL-c concentration (Giugliano and Esposito, 2008) with cardioprotective activity (Aron and Kennedy, 2008).

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