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Protective Effect of Ferulago angulata (Schlecht.) Boiss on N-nitrosodimethylamine induced oxidative stress in rats

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ABSTRACT

The aim of the present study was to investigate the effect of Ferulago angulata (Schlecht.) Boiss. (FASB) extract on mineral (Ca, K, Na) and trace element (Cu, Zn, Se, Co, Fe, Mn, Cr, Pb, Ni, Cd) levels were determined in rat heart tissue, which was oxidatively stressed with N-nitrosodimethylamine (NDMA). Experimental period was continued with Wistar-Albino rats (n:60) in 21 days. Rats were treated with NDMA (10 mg/kg) in first seven days. FASB plant extract was given to the rats each day of the study. The rats were divided into six groups of ten rats each. The mineral and trace element levels of heart tissue were analyzed by using ICP-OES. The results show that NDMA group was significantly lower results than control group with regarding to Zn, Co and Na levels ($p<0.05$, $p<0.01$ and $p<0.05$). On the other hand the NDMA group was also significantly lower results than the FASB (300 mg/kg) group regarding Zn and Co levels ($p<0.05$ and $p<0.05$), whereas the FASB (150 mg/kg) group had increased level of Na according to NDMA group ($p<0.05$). In conclusion, due to the positive effect on significantly alteration in Zn and Co level of heart tissue, it was suggested that the FASB (150, 300 mg/kg) can be used in preventing hearth damage.

Key Words: Ferulago angulata, NDMA, Trace element, minerals

Introduction

The increase in demand for housing and industry related to water infrastructure has led to the search for various supply sources such as the use of wasted water from industrial water treatment plants. The formation of NDMA as a disinfection by-product during water treatment is a major challenge. NDMA is occurs when water is disinfected utilizating chemical substances such as chloramines and chlorine. It is classified as a possible human carcinogen by the International Agency for Cancer Research. (IARC) (Kaserzon et al. 2011).

N-nitrosodimethylamine is a yellow, oily liquid at room temperature. NDMA is a member of n-nitrosoamines. It is highly carcinogenic and toxic (Veena and Manu, 2012). Diet consumption, which is affluent in amine and nitrate, means the risk of the formation of carcinogenic nitrosamines (Ahmad et. al., 2011). Processed milk and milk products, meat and meat products, fish and fish products, baby foods, cereals, beverages, and vegetables and various fruits are among the foods in which NDMA has been detected (Kiziltas et al. 2017). NDMA, contributes to the formation of oxidative stress by increasing free radical production (Ahmad et. al., 2011). The formation of NDMA from these tertiary amines occurs in two steps: dealkylation of the tertiary amine to DMA by reaction with chloramines or hypochlorite and followed by nitration of DMA to NDMA (Lee et al. 2007). NDMA precursors includes tertiary amines, but biological treatment is merely efficient at eliminate DMA (Lee et al. 2008).

Phenolic compounds are existing in high quantity in foods and beverages and are substances which strongly block the formation of N-nitroso compounds (Chung et al. 2002). Those who consume a comparatively high amount of fruits and vegetables carry about 50% less risk of stomach cancer. Therefore, a diet approach for the prevention of N - nitrozamine - induced

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Table 1. Trace Elements (Cu, Cr, Co, Mn, Ni, Fe, Cd, Pb, Se and Zn), Minerals (Ca, K and Na) levels, in control, NDMA, FASB (*), NDMA+ FASB(*), FASB (#) and NDMA+ FASB(#) groups in heart tissue samples

Parameters	Control $\overline{X} \pm SEM$	NDMA $\overline{X} \pm SEM$	*FASB* (*) $\overline{X} \pm SEM$	NDMA+ FASB(*) $\overline{X} \pm SEM$	*FASB* (#) $\overline{X} \pm SEM$	NDMA+ *FASB* (#) $\overline{X} \pm SEM$
Cu (µmol/kg)	6,63 ± 0,27	5,74 ± 0,35	6,15± 0,34	5,87 ± 0,28	6,20 ± 0,40	5,95± 0,37
Zn (µmol/kg)	16,36 ± 0,45^{c}	13,92 ± 0,34^{c,c1}	16,14 ± 0,69	15,19 ± 0,55	16,20 ± 0,58^{c1}	15,55 ± 0,49
Se (µmol/kg)	0,25 ± 0,043	0,21 ± 0,026	0,23 ± 0,027	0,22 ± 0,035	0,24 ± 0,029	0,23 ± 0,046
Fe (µmol/kg)	65,97 ± 2,98	56,59 ± 2,91	59,69 ± 2,80	56,66 ± 3,07	60,30 ± 2,42	59,68 ± 3,27
Cr (µmol/kg)	1,94 ± 0,19	1,77 ± 0,15	1,85 ± 0,10	1,81 ± 0,098	1,86 ± 0,11	1,89 ± 0,14
Mn (µmol/kg)	1,085 ± 0,055	0,91 ± 0,028	0,94 ± 0,079	0,95 ± 0,077	0,96 ± 0,025	0,93 ± 0,026
Co (µmol/kg)	0,11 ± 0,0063^{b}	0,071 ± 0,0078^{b,c}	0,088 ± 0,0077	0,084 ± 0,0057	0,10 ± 0,0071^{c}	0,092 ± 0,0078
Cd (µmol/kg)	0,018 ± 0,0021	0,019 ± 0,0025	0,018 ± 0,0019	0,017 ± 0,0013	0,018 ± 0,0011	0,018 ± 0,0024
Ni (µmol/kg)	1,76 ± 0,12	1,72 ± 0,14	1,67 ± 0,088	1,78 ± 0,11	1,69 ± 0,15	1,73 ± 0,20
Pb (µmol/kg)	0,079 ± 0,011	0,089 ± 0,012	0,077 ± 0,013	0,087 ± 0,026	0,082 ± 0,015	0,080 ± 0,014
Ca (mmol/kg)	0,41 ± 0,013	0,36 ± 0,021	0,38 ± 0,016	0,37 ± 0,017	0,39 ± 0,0076	0,38 ± 0,014
K (mmol/kg)	5,63 ± 0,096	5,17 ± 0,19	5,36 ± 0,20	5,22 ± 0,22	5,33 ± 0,18	5,26 ± 0,21
Na (mmol/kg)	1,95 ± 0,092^{c}	1,49 ± 0,098$^{c, c1}$	1,79 ± 0,091^{c1}	1,53 ± 0,12	1,76 ± 0,089	1,72 ± 0,093

a,a_1,a_2: $p<0.001$, b, b_1: $p<0.01$, c,c_1: $p<0.05$ (different letters, significant differences between groups). *FASB* (*) **(**150 mg/kg**),** *FASB* (#) **(**300 mg/kg**)**

carcinogenesis is proposed (Lee et al. 2006).

FASB is a perennial bush and is 60-150 cm long. It has yellow, umbrella-shaped flowers and thin leaves. It grows in the west of Iran and south of Turkey. The FASB is been used medically as an aphrodisiac and as a helper for digestion. It is been used as a sedative and to gain strength too. In addition, the plant has been reported to have antidiabetic effects and antioxidant (Asghari et. al., 2012). FASB is used as blood sugar decreasing agent in Iran and as food additive to prevent food spoilage (Sajjadi et al. 2016). At the same time, it is traditionally used to treat skin sores, snake bites, headache problems. Modern scientific research has also shown antibacterial, antifungal and anthelmintic effects of *Ferulago* plant (Shahbazi et al. 2016). It is reported in different studies that it can have the feature of anti-cancer (Karimian et. al., 2014). In recent years it was reported that low density lipoprotein (LDL) oxidation is a mechanism that makes lipoproteins atherogenic. Oxidized lipids are associated with the progression of atherosclerosis. The FASB plant prevents the formation of atherosclerosis by lowering serum lipids (Rafieian-kopaei, 2014).

Dietary minerals have importance in the protection of several diseases. Ca, P, Mg, S, K, Cl, Na are major minerals. Major minerals are structural components of tissues and have importance on cellular metabolism and H_2O and acid-base balance. Trace elements are Zn, Fe, Mn, Cu, F, I and Cr and they are essential in smaller amounts for the body (Özcan, 2004). Se, Cu, Mn, Zn participate in the structure of the protective enzymes and the antioxidant state of the body is affected considerably from the diet. Nutritional deficiency leads to weakening of defense mechanisms (Diplock, 1998). Zn, Cu and Mn are

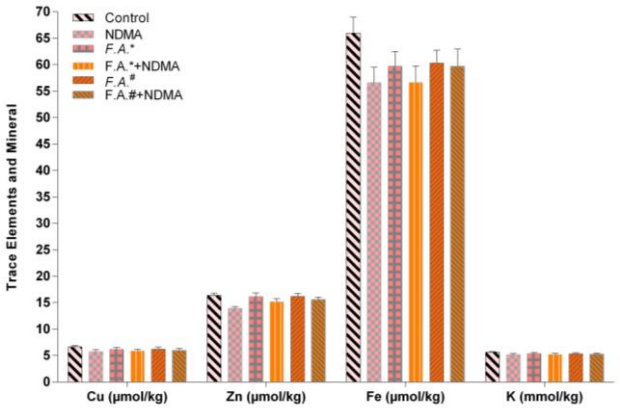

Fig. 1. Trace Element (Cu, Zn, Fe) and Mineral (K) levels in heart tissue samples of control, NDMA, NDMA+150 mg/kg FESB(*),150 mg/kg FESB(*),NDMA+300 mg/kg FESB(#)and 300 mg/kg FESB (#) groups

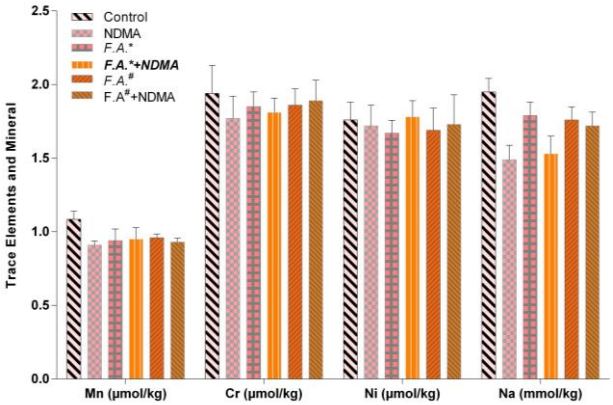

Fig. 2. Trace Element (Se, Co, Pb, Cd) and Mineral (Ca) levels in heart tissue samples of control, NDMA, NDMA+150 mg/kg FESB(*),150 mg/kg FESB(*),NDMA+300 mg/kg FESB(#)and 300 mg/kg FESB (#) groups

required for superoxide dismutase activity. Fe is necessary for catalase activity (Bendich, 1993).

The purpose of this study was to examine of FASB extract on mineral (Ca, K, Na) and trace element (Cu, Zn, Se, Co, Fe, Mn, Cr, Pb, Ni, Cd) levels were determined in rat heart tissue.

Material and Methods

Plant Material: FASB plant used in study was collected from the area between Ozluce and Gecerli villages B9 Gurpinar, VAN, and from 2010m. on 29/07/2012. Identification of the plant species was implemented by Assoc. Prof. Dr. Fevzi OZGOKÇE, instructor at Yuzuncu Yil University Department of Biology, Faculty of Science. Witness plant sample is kept in Herbarium of Van Yuzuncu Yil University with the code of VANF F13982 END. Plant examples were dried in shade without being exposed to sunlight and were kept for analyses.

Animal Material: This study was confirmed with the decision of Ehical Committee Report (No.2014/-03), indicated in the article dated 27/02/2014 and numbered 27552122-110 of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee. Animal material of study was consisted of 60 male wistar-albino rats, which have 200 ± 50 g of body weight. The animals were obtained from Yuzuncu Yil University Research Unit for Animal Experiments. The animals were fed with pellet feed by being free for taking feed and water in rooms which were at 22 ± 2 °C and were lightened rhythmically 12 hours darkness and 12 hours light and held in standard plastic cages.

In study Sixty male wistar-albino rats were used by creating 6 different groups of ten rats each. Group 1: untreated animals were given an equal volume of % 0,9 NaCl. Group 2: animals were administered (10 mg/kg) NDMA. Group 3: animals were given 150 (mg/kg) FASB. Group 4: animals were administered (10 mg/kg) NDMA+ 150 (mg/kg) FASB plant extract. Group 5: animals were administered 300 (mg/kg) FASB plant extract. Group 6: animals were treated with (10 mg/kg) NDMA+ 300 (mg/kg) FASB plant extract. Experimental period was continued within 21 days. Rats were intraperitoneally treated with NDMA (10 mg/kg) in first seven days. FASB plant extract was administered each day of the study by oral gavage to Wistar-albino rats. FASB was dried then extracted in 100 % water and concentrated to dryness. Wistar-albino rats were administered orally with single doses of the extract.

Heart tissue samples which would be used in study were obtained from the rats which were inducted using 10% of ketamine. Tissue samples were washed with physiological water (0,9 NaCl) and were blotted. Heart tissue samples were kept in deepfreeze (-80 °C) by the time biochemical analyses were performed.

Mineral Determination: Determination of the amount of mineral of FASB plant administrated rat heart tissue was determined using dry ashing method. Analyses of Ca, K, Mg, Na, Fe, Mn, Cu, Cr and Zn elements were performed using inductive paired plasma-optical emission spectrometer (ICP-OES).

Statistical Analyses: Results are expressed as $\bar{X}$ ± SEM analysis of variance (ANOVA) was performed, and the statistical comparisons among the groups were carried out with *post- hoc* Tukey's

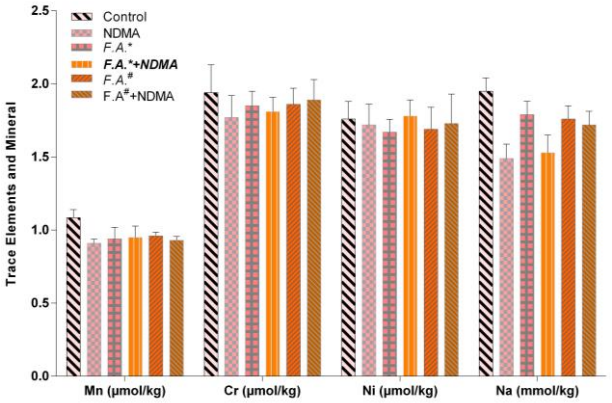

Fig. 3. Trace Element (Mn, Cr, Ni) and Mineral (Na) levels in heart tissue samples of control, NDMA, NDMA+150 mg/kg FESB(*),150 mg/kg FESB(*),NDMA+300 mg/kg FESB(#)and 300 mg/kg FESB (#) groups

test for normally distributed variables, or with nonparametric Benferroni test for non-normally distributed data using a statistical package program (SPSS 22.0 for Windows).

Result

Trace elements and minerals were determined in heart tissue samples. The results show that that NDMA group was significantly decrease results than control group with regarding to Zn, Co and Na levels ($p<0.05$, $p<0.01$ and $p<0.05$). On the other hand the NDMA group was also showed significantly decreased results than the FASB (300 mg/kg) group regarding Zn and Co levels ($p<0.05$ and $p<0.05$), whereas the *FASB* **(**150 mg/kg**)** group had increased level of Na according to NDMA group ($p<0.05$). Moreover, no significant differences Cu, Se, Fe, Cr, Mn, Cd, Ni, Pb, Ca, K levels were observed in the NDMA, 150 mg/kg *FASB*(*), 300 mg/kg *FASB* (#), NDMA+150 mg/kg *FASB*(*),and NDMA+300 mg/kg *FASB*(#) groups, as compared to the control group ($p>0.05$). (Figure 1,2,3).

Discussion

This study was performed to research the effect of FASB extract on trace element (Zn, Cu, Se, Co, Fe, Cr, Mn, Pb, Ni, Cd) and mineral (Ca, Na, K) levels, as well as possible *in vivo* protective properties against NDMA-induced oxidative stress in rats.

NDMA occurs when sewage and drinking waters are disinfected (Kaserzon et.al., 2011). It is quite cancerogenic and toxic (Veena and Manu, 2012). *Ferulago angulata* has been used medically and it was reported that the plant has antidiabetic effects and antioxidant (Asghari et. al., 2012).

There was no study on trace elements in heart tissue in rat groups in which NDMA or *Ferulago angulata* plant was applied. It has shown that the consumption of whole 300 g strawberries, garlic 200 g juice or kale 200 g juice in combination with an amine rich diet containing nitrosatable precursors with 400 mg/day nitrate significant reduce N-nitrosodimethylamine excretion (Chung et al, 2002).

Trace metals is needed in very small quantities. They mostly form metalloproteins attaching to protein in biological system. Metals in metalloproteins constitute some of enzymatic complex have structural functions; in addition, they are benefited to reach target tissue from proteins (Fraga, 2005).

The last amino terminal in structure of the albumin is the region to which transition metals such as cobalt, nickel and copper are bound. It has been found that, due to hypoxia caused by ischemia, particularly because of the increase of reactive oxygen radicals, there is a change in the N-terminal region of the albumin. For this reason, altered albumin's ability to bind cobalt is being reduced (Gençpınar, 2011).

According to the statistical analysis the NDMA administered group showed significantly decrease results than the control group showed with regarding to Co levels ($p<0.01$). NDMA administered group showed significantly lower results than the 300 (mg/kg) FASB plant extract administered group showed with regards to Co level ($p<0.05$). The NDMA administered group showed significantly decrease results than the control group showed with regarding to Na levels ($p<0.05$). Also the NDMA administered group was significantly decrease results than the 150 (mg/kg) FASB plant extract administered group showed with regards to Na level ($p<0.05$). (Table 1)

Zn^{2+} is present in biologic systems in two different states: bound to structural/ regulatory metallo-proteins or histochemically reactive. Especially in recent years, it has been shown that Zn^{2+} is related to 10% of all proteins in cytosol. 70% of the total zinc in the heart muscle is in the cell. That is, 30% in the nucleus and 50% in the cytosol and organelles, the remainder being due to the proteins in the cytosol. Studies in patients with heart failure have shown that serum Zn^{2+} level is low (Tuncay, 2014).

In our study, the NDMA administered group showed significantly decrease results than control

group showed with regarding to Zn levels $p<0.05$). Similarly the NDMA administered group showed significantly lower results than the 300 (mg/kg) FASB plant extract administered group showed with regards to Zn level ($p<0.05$). Zn is an exogenous nutritional antioxidant. It is found in soil, water and vegetables, seafood, liver and yeast. It forms the active site of many enzymes (Rao et al. 2011).

As a result, it was determined that rat groups heart tissue mineral levels which FASB plant was administered were quite high. Therefore, we have the opinion that FASB plant can be used in prevention and cure of various diseases, caused by free radicals and particularly chemicals which contributes to oxidative stress formation by production free radicals like NDMA. Also the plant can be used in preventing and treatment of heart damages. Our data indicated that an inhibition of N-nitrosamine formation by trace elements (Zn, Co) in FASB plant.

In conclusion**,** owing to the positive effect on significantly alteration in Zn and Co level of heart tissue, it was suggested that the *FASB* **(**150, 300 mg/kg**)** can be used in preventing hearth damage.

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