Cardioprotective effects of the garlic (Allium sativum) in sodium fluoride-treated rats

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Abstract

**Background:** Excessive intake of fluoride may result in the development of cardiotoxicity in the rats. The objective of this study was to investigate the possible cardioprotective effect of the garlic (G) on sodium fluoride (NaF)-treated rats.

**Methods:** Twenty-four male albino rats (100–120 g), 2 months old, were equally divided into control, NaF, G, and NaF + G groups. Group 1 was control group, the animals without any treatment. Group 2 was administrated with NaF orally (10 mg/kg BWT) daily. Group 3 received orally G alone (63 mg/kg BWT) daily. Group 4 was administrated with NaF + G at the same time (with the same previous doses) daily. The experimental period was for 4 weeks.

**Results:** NaF significantly elevated the levels of serum creatinine kinase (CK), creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and cardiac troponin I (cTnI). Also, there was a significant increase in the total cholesterol (TC), triglycerides (TAG), low-density lipoprotein (LDL-c) fractions, and the atherogenic effect (the mean ratios of TC/LDL-c and LDL-c/ high-density lipoprotein HDL-c), whereas a significant decrease in HDL-c occurred in the NaF-treated group compared with the control animals. The treatment with G+NaF ameliorated all the biochemical parameters tested.

**Conclusion:** These results indicate that garlic has a cardioprotective effect against NaF cardiotoxicity.

**Keywords:** Sodium fluoride, Garlic, Cardioprotective, Lipid profile

Introduction

Fluoride is widely used in the modern preventive dentistry, as it has cariostatic properties. The major sources of fluoride intake are water, milk derivatives, fish, chicken, and toothpaste and other products containing fluoride (Levy, Kiritsy, & Warren, 1995). Fluoride has been usually added to toothpastes and mouth rinses (Chachra, Vieira, & Grynpas, 2008). Also, fluoride is a common ingredient of drinking water and foodstuffs (Svarc-Gajić, Stojanović, Vasiljević, & Kecojević, 2013) and commonly used in a lot of industrial works, like the production of aluminum, ceramics, phosphoric acid, phosphate fertilizers, and brick and steel (Ameeramja et al., 2015). Moreover, sodium fluoride is a useful phosphatase inhibitor. The American Association of Poison Control Centers reported that of the cases of fluoride intoxication, 68% were related to fluoride dentifrice ingestion, 17% to fluoride mouth rinses, and 15% to fluoride supplements. Children younger than 6 years of age account for more than 80% of reports of suspected over ingestion (Shulman & Well, 1997). The effects of chronic fluoride ingestion related to the dose and duration and several other factors such as nutritional status, renal function, and the interactions with other elements (Ponikvar, 2008). Few records are ready-made about the concentration of fluoride in the River Nile. Nile Basin Initiative (NBI) (2005) reported that the fluoride concentration in the River Nile from Aswan to Cairo ranges between 0.391 and 1.969 mg/L and between 0.259 and 0.487 in the main irrigation canals. Reports of the Egyptian Environmental Affairs Agency showed that fluoride concentration in the River Nile at different government monitoring stations ranges between 0.11 and 0.56 mg/L (EEAA, 2009). The concentration of fluoride in drinking water should be between 1.0 and 1.5 mg/L, according to the World Health Organization (WHO, 1993) guidelines. Chronic toxicity of fluoride is more common than acute...
toxicity. Excessive intake of fluoride may result in skeletal and dental fluorosis in humans and animals (Irigoyencamacho, García, Mejía, & Huiziar, 2015) and changes in the structure and function of soft tissues, including liver (Mukhopadhyay, Srivastava, & Chattopadhyay, 2015), brain (Zheng, Sun, Ke, Ouyang, & Zhang, 2016), lung (Shanmugam, Selvaraj, & Poomalai, 2016), spleen (Kuang et al., 2017), thymus (Stawiarska-Pięta et al., 2013), heart (Oyagbemi et al., 2017), intestine (Luo et al., 2013), and reproductive organs (Wei, Luo, Sun, Wang, & Wang, 2016). Also, the kidney is very sensitive to fluoride toxicity (Song et al., 2014). The animals exposed to fluoride chronically have significantly high levels of both fluoride and calcium in the aorta (Tuncel, 1984) and the heart (Susheela & Kharb, 1990), while acute fluoride intoxication leads to the progressive fall in arterial blood pressure causing cardiovascular damage (Strubelt, Iven, & Younes, 1982), and the prolonged ingestion of fluoride may directly induce histopathological and biochemical changes in myocardial tissue (Ciccek, Aydin, Akdogan, & Okutan, 2005). The previous studies proved that fluoride had contrary effects on hematological and biochemical parameters in rats (Agha, El-Badry, Hassan, & Abd Elraouf, 2012) and humans (Ravichandran, Chattopadhyay, Gangopadhyay, & Saiyed, 2012). Also, other studies have reported that cardiac failure patients have high levels of fluoride in the blood. Acute fluoride toxicity signs include vomiting, nausea, drop in blood calcium, signs of muscle tetany, abdominal cramping, increasing hypocalcaemia, hyperkalemia, leading coma, convulsions, and cardiac arrhythmias. The death from excessive fluoride ingestion may occur within 4 h; if the individual survives for 24 h, the prognosis may be good (Norman & Arden, 1991).

Traditionally, the garlic herb (Allium sativum, family: Liliaceae) has both medicinal and nutritional valuable since ancient times with various biological effectiveness (Bradley, Organ, & Lefer, 2016). Preparations of the garlic have been used in the prevention and treatment of cardiovascular and many metabolic diseases such as hyperlipidemia, atherosclerosis, arrhythmia (Khan, Hassan, Sarder, & Anjum, 2008), diabetes thrombosis, and hypertension (Banerjee & Maulik, 2006). Further, garlic was reported to have cardioprotective, antioxidant, anti-neoplastic, and antimicrobial properties (Rahman & Lowe, 2006), and it has significant antiarrhythmic influence in both ventricular and supraventricular arrhythmias (Reitz, Isence, Strobach, Makdessi, & Jacob, 1993).

The present research was designed to evaluate the cardioprotective effect of the garlic against the cardiotoxicity induced by sodium fluoride in male rats, by estimating some biochemical markers of cardiac injury, serum lipid profile, and liver enzymes. The garlic contains various components including proteins, carbohydrates, fats, minerals, and vitamins (Cobas, Soria, Martinez, & Villamiel, 2010). Also, many vitamins are present in garlic which include vitamin A; various kinds of vitamin B, such as thiamine, nicotinic acid, and riboflavin; and vitamins E and C. Sulfur compounds present also in the garlic related to its biological and pharmacological effects (Lanzotti, 2006). Some of these organo-sulfur compounds are alilin, allicin, vinylthiinnes, ajepone, allylpropyl disulfide, S-allylmercaptocystein, sallylcysteine, diallyl trisulfide, and others (Sarkar et al., 2006).

Materials and methods
Chemicals and drugs
Sodium fluoride (NaF) was used in this experiment, obtained from Sigma Chemical Company. The animals received orally NaF at a dose of 10 mg/kg BWT according to Abdel-Wahab (2013) daily for 4 weeks. One gram of NaF was dissolved in 100 ml normal saline solution (0.9% NaCl). Every rat received orally 1 ml of the prepared solution equals 10 mg/kg BWT.

Garlic was obtained from Sekim Company for Pharmaceutical Industries. Garlic is available in the form of tablets. Each enteric coated tablet contains dried garlic powder 300 mg given three times every day for human. The dose of garlic (63 mg/kg BWT) was calculated according to the equivalent therapeutic dosages of human-mouse conversion factor by Paget and Barnes (1964) and was given orally for the desired period (4 weeks). One garlic tablet was diluted in 40 ml normal saline solution (0.9% NaCl). Every rat received orally 1 ml of diluted tablet equals 63 mg/kg BWT.

Nutritional value and properties of garlic. Values expressed per 100 g of raw garlic were detected by Cobas et al. (2010)

| Properties | Values | Minerals | Values | Vitamins | Values |
|------------|--------|----------|--------|----------|--------|
| Energy     | 119 kcal | Potassium | 446 mg | Thiamin (vit. B1) | 0.16 mg |
| Moisture   | 70% | Phosphorus | 134 mg | Riboflavin (vit. B2) | 0.02 mg |
| Protein    | 4.3 g | Magnesium | 24.1 mg | Niacin (vit. B3) | 1.02 mg |
| Carbohydrate | 24.3 g | Sodium | 19 mg | Puridoxin (vit. B6) | 0.32 mg |
| Fiber      | 1.2 g | Calcium | 17.8 mg | Folic acid | 4.8 μg |
| Fat        | 0.23 g | Iron | 1.2 mg | Ascorbic acid (vit. C) | 14 mg |
| Alcohol    | 0 g | Zinc | 1.1 mg | Carotenoids (β-carorenes) | 5 μg |
| Ash        | 2.3% | Iodine | 4.7 μg | Vitamin A | Traces |
| PH         | 6.05 | Selenium | 2 μg | Vitamin E (tocopherols) | 0.011 μg |
| Acidity    | 0.172% | | | | |

| PH         | 6.05 | Selenium | 2 μg | Vitamin E (tocopherols) | 0.011 μg |
Animals

Twenty-four adult male albino rats, each weighing 100–120 g (2 months old), were purchased from the farm of the National Organization for Drug Control and Research, Giza, Egypt. The rats were preserved under common and controlled laboratory conditions of relative humidity (55 ± 5%) and temperature (25 ± 5°C), with a natural light/dark cycle and free entrance to slandered diet and water ad libitum. The rats for the study were humanely handled in accordance with the ethics and regulation guiding the use of research animals as approved by the university.

Experimental design

Study protocol

After 2 weeks of acclimatization, the rats were divided randomly into four groups each of six rats as follow:

Group I (control): The animals without any treatment.
Group II: The animals received orally NaF at a dose of 10 mg/kg BWT.
Group III: The animals were given orally G alone at a dose of 63 mg/kg BWT.
Group IV: The animals received orally NaF and G at the same time (with the same previous doses).

After the end of treatment, rats were fasted overnight (24 h).
Then, the animals in each group were anesthetized with diethyl ether. After that, rats were sacrificed by cervical dislocation and the blood samples were collected; then, the sera were obtained by blood centrifugation at 3000 rpm for 15 min. Blood sera were collected and immediately stored at −20 °C until the time of analysis.

Biochemical assays

Triglycerides (TAG) were estimated according to the method of Buccolo and David (1973). Total cholesterol (TC) was determined in the serum according to the method of Allain, Poon, Clen, Richmond, and Fu (1974). High-density lipoprotein cholesterol (HDL-c) was estimated in the serum as described by Kostener (1977). Low-density lipoprotein cholesterol (LDL-c) was calculated according to the method of Glatter (1984), using the following equation. LDL-c = [(total cholesterol) – (triglyceride/5) – (HDL – chol)]. Ratios of TC/HDL-c (risk factor 1) and LDL-c/HDL-c (risk factor 2) were calculated.

Serum was used for estimation of CK according to Young (2001), CK-MB according to Urdal and Landaas (1979), and LDH according to Scientific Committee (1982). Serum AST and ALT activities were measured calorimetrically according to the method of Reitman and Frankel (1957).

Statistical analysis

Statistical analysis was carried out using one-way analysis of variance (ANOVA) to assess the significant differences between treatment groups. Duncan’s multiple range test was used to estimate the significant differences between means. The results are expressed as mean ± standard error (SE). The p values were considered statistically significant at p < 0.05.

Results

The administration of sodium fluoride caused a significant increase in serum total creatinine kinase (CK), creatine kinase–MB (CK-MB ), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), Aalanine aminotransferase (ALT), and cardiac troponin I (cTnI) levels in rats compared with the control group. Regarding the garlic-treated group, the obtained results showed a non-significant change in the measured parameters as compared with the control group. Also, the garlic administration significantly alleviated the rise in the serum levels of these parameters parallel to the sodium fluoride-treated group (Table 1).

Table 2 illustrated the effect of sodium fluoride and garlic administration on serum lipid profile, including TC, TAG, HDL-c, and LDL-c concentrations, and the atherogenic effect including the ratios of TC/HDL and LDL/HDL in different groups. Sodium fluoride treatment significantly increased the serum TC, TAG, LDL-c, TC/HDL, and LDL/HDL ratios in sodium fluoride-treated animals parallel to the control group that indicates hypertriglyceridemia and hypercholesterolemia, whereas the serum concentration of HDL-c is significantly decreased in sodium fluoride-treated rats compared with the control ones. The treatment with garlic only showed a significant decrease in TC and TAG concentrations recording – 13.96% and – 17.65%, respectively, below the control value. However, there was a non-significant change in HDL-c, LDL-c, TC/HDL, and LDL/HDL ratios as compared to the control group. The treatment with sodium fluoride and the garlic together significantly improved the tested parameters in rats. The treatment with garlic only showed a significant decrease in TC and TAG concentrations recording – 13.96% and – 17.65%, respectively, below the control value. However, there was a non-significant change in HDL-c, LDL-c, TC/HDL, and LDL/HDL ratios as compared to the control group. The treatment with sodium fluoride and the garlic together significantly improved the tested parameters in rats. TC, TAG, and LDL-c concentrations and TC/HDL and LDL/HDL calculated ratios were significantly decreased compared to the sodium fluoride-treated group. At the same time, the HDL-c concentration showed an increase compared with the sodium fluoride-treated group.

Discussion

The present study reports the ability of the garlic (Allium sativum) to normalize serum cardiac biomarkers and lipid profile in sodium fluoride-treated male rats.
Fluoride affects cardiovascular function (Bera et al., 2007), involved in inflammatory and degenerative changes in the liver (Anamika, Komal, & Ramtej, 2012), and results in abnormal metabolic function in different species (Kotodziezyk, Put, & Gizela, 2000). Metabolically damaged myocardium has high concentrations of diagnostic biomarkers for myocardial infarction; it resulted in the leakage of its content into the extracellular fluid (Upaganlawar, Gandhi, & Balaraman, 2009). Serum levels of creatinine kinase (CK), creatine kinase–MB (CK-MB), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and cardiac troponin I (cTnI) are important diagnostic markers of myocardial damage. Creatine kinase (CK) enzyme is present in the muscles, brain, colon, and urinary bladder. Its physiological role is to keep phosphorylated creatine that is used to maintain ATP levels that depleted during muscle contraction. CK consists of two subunits existing in three molecular forms, namely CK-MM, CK-MB, and CK-BB. CK-MB predominates in the heart muscle. Serum total CK activity and CK-MB concentration rise in parallel following myocardial injury. Jaffe, Ravkilde, and Roberts (2000) reported that serum CK-MB is considerably more specific for myocardial damage. LDH is an enzyme present in all cells of the body with highest concentrations in the heart, liver, muscles, kidney, lungs and erythrocytes. Feng, Chen, Chiu, George, and Chakrabarti (2008) have suggested that the peak rise in LDH is proportional to the extent of injury to the myocardial tissue. Cardiac troponin I (cTnI) is a protein specific to the tissue of the heart. It is a specific biochemical marker of myocardial cell necrosis, acute myocardial infarction, and cardiotoxicity induced by the drugs (Gaze & Collinson, 2005). Aminotransferases (AST and ALT) mediate the reactions of aminotransfer catalysis and are considered as markers for liver injury diagnosis.

In the present study, it was observed that sodium fluoride induced elevated serum levels of CK, CK-MB, LDH, AST, ALT, and cTn I, whereas the administration of the garlic to rats and the combination between the garlic and sodium fluoride caused a significant decrease in these cardiac biomarkers (CK, CK-MB, LDH, troponin, AST, and ALT enzymes) in comparison with those of the sodium fluoride-treated group. These results are in agreement with Yildirim et al. (2018), who reported an increase in the levels of CK, LDH, AST, ALT, and cTn I in the sodium fluoride-treated rats. Also, reportedly, garlic extract was able to obtain the same result in isoproterenol-induced myocardial toxicity in rats (Avula, Asdaq, & Asad, 2014). The present results are inconsistent with those of some other research workers, but they use gentamicin instead of sodium fluoride (Gomaa, Abdelhafez, & Aamer, 2018). These findings

**Table 1** Effect of the garlic on sodium fluoride-induced changes in serum CK, CK-MB, cTN 1, LDH, AST, and ALT

| Parameters Groups | Control | Sodium fluoride | Percentage | Garlic | Percentage | Sodium fluoride + Garlic | % | p value |
|------------------|---------|-----------------|------------|--------|------------|--------------------------|---|---------|
| Total CK         | 128.7± 1.94 | 744.8± 19.1 | 478.7 | 132.3± 1.84 | 2.8 | 340.5± 7.61 | 164.6 | * |
| CK-MB            | 280.17± 1.64 | 458.4± 1.91 | 63.6 | 279± 0.95 | − 0.42 | 300.5± 2.3 | 7.3 | * |
| cTN 1            | 0.3± 0.03 | 0.8± 0.04 | 166.7 | 0.2± 0.03 | − 33.33 | 0.4± 0.1 | 33.33 | * |
| LDH              | 137.5± 1.2 | 173.8± 1.96 | 26.4 | 139.3± 0.9 | 1.31 | 147.2± 1.7 | 7.1 | * |
| ALT              | 79.8± 1.14 | 137.2± 1.5 | 71.93 | 84.3± 1.5 | 5.7 | 98.2± 0.95 | 23.1 | * |

*Data are represented as (mean ± SE). Values with different letters within the same row significantly differed at (p < 0.05)

*% D: Percentage difference [(treated value − control value)/control value] × 100

**Table 2** Effect of the garlic on sodium fluoride-induced changes in serum total cholesterol (TC), triglycerides (TAG), high-density lipoprotein (HDL-C) cholesterol, low-density lipoprotein (LDL-C) cholesterol, and the calculated ratios of TC/HDL and LDL/HDL

| Parameters Groups | Control | Sodium fluoride | Percentage | Garlic | Percentage | Sodium fluoride + garlic | % | p value |
|------------------|---------|-----------------|------------|--------|------------|--------------------------|---|---------|
| TC               | 74.15± 1.65 | 132± 1.8 | 78.02 | 63.8± 2.3 | − 13.9 | 91± 1.7 | 22.7 | * |
| Trigly           | 79.33± 2.2 | 36.14 | 65.3± 1.84 | − 17.6 | 90.3± 2.5 | 13.9 | * |
| HDL-c            | 41.2± 1.1 | 20.9 | 38.8± 1.6 | − 5.8 | 38.5± 1.5 | − 6.6 | * |
| LDL-c            | 16± 0.97 | 108± 2.2 | 36.14 | 65.3± 1.84 | − 17.6 | 90.3± 2.5 | 13.9 | * |
| TC/HDL-c         | 1.82± 0.05 | 21.7± 0.02 | 125.3 | 1.7± 0.1 | − 66 | 2.3± 0.1 | 26.4 | * |
| LDL-c /HDL-c     | 0.4± 0.03 | 1.2± 0.1 | 200 | 0.3± 0.02 | − 12.5 | 0.7± 0.1 | 75 | * |

*Data are represented as (mean ± SE). Values with different letters within the same row significantly differed at (p < 0.05)

*% D: Percentage difference [(treated value − control value)/control value] × 100
indicate the onset of myocardial necrosis and the damage of the cell membrane by sodium fluoride that caused the release of these biomarkers from the heart to blood. Since any serious insult to the heart muscle will enhance the release of CK-MB, LDH, and AST enzymes into the serum of animals according to Badole et al. (2015). Also, an elevated cTn I level indicates cardiac injury including acute perimyocarditis and acute coronary injury including acute pulmonary embolism, acute heart failure, and tachycardia (Vasudevan, Sreekumari, & Kannan, 2013). The reduction of all above cardiac biomarkers in the serum by garlic indicates that it could maintain membrane integrity and limit the leakage of these biomarkers by protecting the cell membrane from the destructive effect of free radicals and also by inhibiting the oxidative modification of LDL as well by balancing lipid profile (Rahman & Lowe, 2006). This may be attributed to the phytochemicals in the garlic such as S-allylcysteine (SAC), sulfur metabolites, and S Allylmercaptocysteine (SAMC), which have powerful antioxidant activity (Asdaq, Inamdar, & Asad, 2010). Also, allicin was easily degraded into organic diallyl polysulfide in the presence of thiols, to provide H2S that protects the heart (Bradley et al., 2016).

Hyperlipidemia has an important role in the development of atherosclerosis and cardiovascular diseases (Hassarajani, Souza, & Mengi, 2007). A significant rise in the serum total cholesterol (TC), triglycerides (TAG), and low-density lipoprotein (LDL-c) fraction and the atherogenic effect (the mean ratio of TC/LDL-c and LDL-c/HDL-c), with the decrease in the level of high-density lipoprotein (HDL-c), were obtained in the sodium fluoride-treated group compared to the control group. These changes concerning lipid profile come in harmony with Abdel-Wahab (2013), who reported that the oral administration of sodium fluoride induced a significant increase in the levels of total lipids, TAG, and TC, and these alterations in the lipid profile may be attributed to high levels of sodium fluoride leading to its aggregation in the liver causing the disturbance of lipid metabolism and the elevation of lipid profile. Also, the treatment with sodium fluoride leads to an increase in lipid peroxidation and membrane integrity loss, causing altered lipid metabolism and hyperlipidemia.

One of the most important factors responsible for the increase in serum triglycerides and cholesterol is abnormal enzyme activities. It appears that sodium fluoride prevents phospholipases, lipases, unspecific esterases, and pyro-phosphatase (Grucka-Mamczar et al., 2004). A potent positive relation was well recorded between developing ischemic heart disease and increased serum LDL cholesterol level (Parikh, Tripathi, Shah, Ghorri, & Goyal, 2015). The supplementation with the garlic alleviated the previously mentioned changes in the lipid profile. The mechanism may be include inhibiting enzymes involved in cholesterol synthesis and deactivation of HMG-CoA reductase and also reducing the hepatic activities of lipogenic enzymes such as malic enzyme, fatty acid synthase, glucose-6 phosphate dehydrogenase (Yeh & Liu, 2001), which also contain high levels of telurium and selenium compounds, which contribute to the overall block in cholesterol synthesis by inhibiting squalene monoxygenase that plays an important role in the overall regulation of cholesterol biosynthesis (Larner, 1995). With respect to cholesterol-lowering property of garlic, it has been suggested that some constituents of garlic may act as inhibitors for some enzymes, like hydroxyl methyl glutaryl CoA reductase, which participate in the cholesterol synthesis (Gebhardt & Beck, 1996). The reduction in LDL level by garlic may be due to the inhibition of LDL oxidation (Lau, 2001).

**Conclusion**

From this study, the garlic administration caused a significant reduction in the biochemical markers of cardiac function as seen in the decrease of serum total CK, CK-MB, cTn I, LDH, AST, and ALT levels and lipid profile that is elevated by sodium fluoride. So it can be concluded that garlic can ameliorate sodium fluoride-induced cardiac malfunction and altered lipid profile in rats.

**Abbreviations**

ALT: Alanine aminotransferase; ANOVA: Analysis of variance; AST: Aspartate aminotransferase; CK: Creatinine kinase; CK-MB: Creatine kinase–MB; cTnI: Cardiac troponin I; G: Garlic; HDL-c: High-density lipoprotein; LDL: Lactate dehydrogenase; LDL-c: Low-density lipoprotein; NaF: Sodium fluoride; NB: Nile Basin Initiative; SE: Standard error; TAG: Triglycerides; TC: Total cholesterol

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**Authors’ contributions**

ESA suggested, planned, and designed the study; performed the data analysis; and wrote and edited the manuscript. ONA contributed to manuscript writing, correction, and preparation. Both authors read and approved the final manuscript.

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**Availability of data and materials**

Data will not be shared, only by request from the corresponding author.

**Ethics approval and consent to participate**

The rats for the study were humanely handled in accordance with the ethics and regulation guiding the use of research animals as approved by the university.

**Consent for publication**

Not applicable.
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