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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 cardiotixicity 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), Aalanine 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, Cardioprtoctive, 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 (Švarc-Gajić, Stojanović, Vasiljević, & Kecojević, 2013) and commonly used in a lot of industrial workouts, 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

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toxicity. Excessive intake of fluoride may result in skeletal and dental fluorosis in humans and animals (Irigoyencamacho, García, Mejía, & Huizar, 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 (Cicek, 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: Lilliaceae) 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, 2002). Further, garlic was reported to have cardioprotective, antioxidant, antineoplastic, 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 aliin, allicin, vinyldithiines, ajoene, 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%				

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. 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.

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	$128.7^{a} \pm 1.94$	$744.8^{b} \pm 19.1$	478.7	$132.3^{a} \pm 1.84$	2.8	$340.5^{c} \pm 7.61$	164.6	*
CK-MB	$280.17^{a} \pm 1.64$	$458.4^{b} \pm 1.91$	63.6	$279^{a} \pm 0.95$	− 0.42	$300.5^{c} \pm 2.3$	7.3	*
cTN 1	$0.3^{a} \pm 0.03$	$0.8^{b} \pm 0.04$	166.7	$0.2^{a} \pm 0.03$	− 33.33	$0.4^{ac} \pm 0.1$	33.33	*
LDH	$357.3^{a} \pm 3.5$	$544.1^{b} \pm 2.8$	52.3	$347.7^{a} \pm 1.81$	− 2.7	$444.2^{c} \pm 16.62$	24.3	*
AST	$137.5^{a} \pm 1.2$	$173.8^{b} \pm 1.96$	26.4	$139.3^{a} \pm 0.9$	1.31	$147.2^{c} \pm 1.7$	7.1	*
ALT	$79.8^{a} \pm 1.14$	$137.2^{b} \pm 1.5$	71.93	$84.33^{a} \pm 1.5$	5.7	$98.2^{c} \pm 0.95$	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

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 (Kotodziejezyk, 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), Aalanine aminotransferase (ALT), and cardiac troponin I (cTn I) are important diagnostic markers of myocardiam 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 troponin 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 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	Percentage	*p* value
TC	$74.15^{a} \pm 1.65$	$132^{b} \pm 1.8$	78.02	$63.8^{c} \pm 2.3$	− 13.9	$91^{d} \pm 1.7$	22.7	*
Trigly	$79.33^{a} \pm 2.2$	$108^{b} \pm 2.2$	36.14	$65.3^{c} \pm 1.84$	− 17.6	$90.33^{d} \pm 2.5$	13.9	*
HDL-c	$41.2^{a} \pm 1.1$	$32.6^{b} \pm 1.01$	− 20.9	$38.8^{a} \pm 1.6$	− 5.8	$38.5^{a} \pm 1.5$	− 6.6	*
LDL-c	$16^{a} \pm 0.97$	$38.5^{b} \pm 2.01$	140.63	$13.2^{a} \pm 1.14$	− 17.5	$24.7^{c} \pm 1.63$	54.4	*
TC/HDL-c	$1.82^{a} \pm 0.05$	$4.1^{b} \pm 0.2$	125.3	$1.7^{a} \pm 0.1$	− 6.6	$2.3^{c} \pm 0.1$	26.4	*
LDL-c /HDL-c	$0.4^{a} \pm 0.03$	$1.2^{b} \pm 0.1$	200	$0.35^{a} \pm 0.02$	− 12.5	$0.7^{c} \pm 0.1$	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 1 level indicates cardiac injury including acute perimyocarditis and acute coronary injury including acute pulmonary embolism, acute heart failure, and tachycardia (Vasudevan, Sreekumari, & Kannnan, 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 Sallylmercaptocysteine (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, Ghori, & 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 tellurium and selenium compounds, which contribute to the overall block in cholesterol synthesis by inhibiting squalene monooxygenase 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: Aalanine 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; LDH: Lactate dehydrogenase; LDL-c: Low-density lipoprotein; NaF: Sodium fluoride; NBI: 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.

Competing interests
The authors declare that they have no competing interests.

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References
Abdel-Wahab, W. M. (2013). Protective effect of thymoquinone on sodium fluoride-induced hepatotoxicity and oxidative stress in rats. *The Journal of Basic & Applied Zoology*, *66*, 263–270.

Agha, F. E., El-Badry, M. O., Hassan, D. A. A., & Abd Elraouf, A. (2012). Role of vitamin E in combination with methionine and L-carnosine against sodium fluoride-induced hematological, biochemical, DNA damage, histological and immunohistochemical changes in pancreas of albino rats. *Life Science Journal*, *9*(2), 1260–1275.

Allain, C. C., Poon, L. S., Clen, C. S. G., Richmond, W., & Fu, P. C. (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, *20*, 470–475.

Ameeramja, J., Panneerselvam, L., Govindarajan, V., Jeyachandran, S., Baskaralingam, V., & Perumal, E. (2015). Tamarind seed coat ameliorates fluoride induced cytotoxicity, oxidative stress, mitochondrial dysfunction and apoptosis in A549 cells. *Journal of Hazardous Materials*, *301*, 554–565.

Anamika, J. H. A., Komal, S., & Ramtej, J. V. (2012). Effects of sodium fluoride on DNA, RNA and protein contents in liver of mice and its amelioration by Camellia sinensis. *Acta Poloniae Pharmaceutica ñ Drug Research*, *69*(3), 551–555.

Asdaq, S. M. B., Inamdar, M. N., & Asad, M. (2010). Pharmacodynamic interaction of garlic with propranolol in ischemia-reperfusion induced myocardial damage. *Pakistan Journal of Pharmaceutical Sciences*, *23*, 42–47.

Avula, P. R., Asdaq, S. M., & Asad, M. (2014). Effect of aged garlic extract and s-allyl cysteine and their interaction with atenolol during isoproterenol induced myocardial toxicity in rats. *Indian Journal of Pharmacology*, *46*, 94–99.

Badole, S. L., Chaudhari, S. M., Jangam, G. B., Amit, G. B., Kandhare, A. D., & Bodhankar, S. L. (2015). Cardioprotective activity of Pongamia pinnata in streptozotocin-nicotinamide induced diabetic rats. *Bio Med Research International*, *403291*, 8 https://doi.org/10.1155/2015/403291.

Banerjee, S. K., & Maulik, S. K. (2002). Effect of garlic on cardiovascular disorders: A review. *Nutrition Journal*, *1*, 4.

Bera, I., Sabatini, R., Auteri, P., Flace, P., Sisto, G., Montagnani, M., … Cagiano, R. (2007). Neurofunctional effects of developmental sodium fluoride exposure in rats. *European Review for Medical and Pharmacological Sciences*, *11*, 211–224.

Bradley, J. M., Organ, C. L., & Lefer, D. J. (2016). Garlic-derived organic polysulfides and myocardial protection. *The Journal of Nutrition*, *146*(2), 403–409.

Buccolo, G., & David, H. (1973). Quantitative determination of serum triglycerides by the use of enzymes. *Clinical Chemistry*, *19*, 419–432.

Chachra, D., Vieira, A. P. G. F., & Grynpas, M. D. (2008). Fluoride and mineralized tissues. *Critical Reviews in Biomedical Engineering*, *36*(2-3), 183–223.

Cicek, E., Aydin, G., Akdogan, M., & Okutan, H. (2005). Effects of chronic ingestion of sodium fluoride on myocardium in a second generation of rats. *Human and Experimintal Toxicology*, *24*(2), 79–87.

Cobas, A. C., Soria, A. C., Martinez, M. C., & Villamiel, M. (2010). *A comprehensive survey of garlic functionality*, (pp. 1–60). Nova Science Publishers, Inc. ISBN 978-1-60741-642-5.

Committee, S. (1982). Recommandations pour la mesure de la concentration catalyique de lactate deshidrogenasedans le serum human a 30 oc. *Annales De Biologie Clinique*, *40*, 87–164.

EEAA (Egyptian Environmental Affairs Agency) (2009). *Egypt state of the environment report 2008*. Egypt: Ministry of State for Environmental Affairs.

Feng, B., Chen, S., Chiu, J., George, B., & Chakrabarti, S. (2008). Regulation of cardiomyocyte hypertrophy in diabetes at the transcriptional level. American journal of physiology. *Endocrinology and metabolism*, *294*, 1119–1126.

Gaze, D. C., & Collinson, P. O. (2005). Review: Cardiac troponins as biomarkers of drug- and toxin-induced cardiac toxicity and cardioprotection. *Expert Opinion on Drug Metabolism &Toxicology*, *1*(4), 715–725.

Gebhardt, R., & Beck, H. (1996). Differential inhibitory effects of garlic-derived organosulfur compounds on cholesterol biosynthesis in primary rat hepatocyte cultures. *Lipids*, *31*, 1269–1276.

Glatter, T. R. (1984). Hyperlipidemia, what is normal, who should be treated and how? *Journal of Post-graduate Medecine*, *76*(6), 49–55.

Gomaa, A. M. S., Abdelhafez, A. T., & Aamer, H. A. (2018). Garlic (Allium sativum) exhibits a cardioprotective effect in experimental chronic renal failure rat model by reducing oxidative stress and controlling cardiac Na+/K+-ATPase activity and Ca2+ levels. *Cell Stress and Chaperones*, *23*(5), 913–920.

Grucka-Mamczar, E., Birkner, E., Kasperczyk, S., Kasperczyk, A., Chlubek, D., & Samujlo, D. (2004). Lipid balance in rats with fluoride induced hyperglycemia. *Fluoride*, *37*, 195–200.

Hassarajani, S., Souza, T. D., & Mengi, S. A. (2007). Efficacy study of the bioactive fraction (F-3) of Acorus calamus in hyperlipidemia. *Indian Journal of Pharmacology*, *39*, 196–200.

Irigoyencamacho, M. E., García, P. A., Mejía, G. A., & Huizar, A. R. (2015). Nutritional status and dental fluorosis among school children in communities with different drinking water fluoride concentrations in a central region in Mexico. *Science of the Total Environment*, *541*, 512–519.

Jaffe, A. S., Ravkilde, J., & Roberts, R. (2000). It's time for a change to a troponin standard. *Circulation*, *102*, 1216–1220.

Khan, S. H., Hassan, S., Sarder, R., & Anjum, M. A. (2008). Effects of dietary garlic powder on cholesterol concentration in native desi laying hens. *American Journal of Food Technology*, *3*(3), 207–213.

Kostener, C. M. (1977). Enzymatic determination of cholesterol high density lipoprotein fraction prepared by polyanion precipitation. *Clinical Chemistry*, *22*, 695.

Kotodziejezyk, L., Put, A., & Gizela, P. (2000). Liver morphology and histochemistry in rats resulting from ingestion of sodium selenite and sodium fluoride. *Fluoride*, *33*, 6–16.

Kuang, P., Deng, H. D., Cui, H. M., Chen, L., Fang, J., Zuo, Z. C., … Zhao, L. (2017). Sodium fluoride (NaF) causes toxic effects on splenic development in mice. *Oncotarget*, *8*(3), 4703–4717.

Lanzotti, V. (2006). The analysis of onion and garlic. *Journal of Chromatography A*, *1112*, 3–22.

Larner, A. (1995). How does garlic exert its hypocholesterolaemic action? The tellurium hypothesis. *Medical Hypotheses*, *44*, 295–297.

Lau, B. H. (2001). Suppression of LDL oxidation by garlic. *The Journal of Nutrition*, *131*, 9555–9585.

Levy, S. M., Kiritsy, M., & Warren, J. J. (1995). Sources of fluoride intake in children. *Journal of Public Health Dentistry*, *55*, 39–52.

Luo, Q., Cui, H. M., Peng, X., Fang, J., Zuo, Z. C., Deng, J. L., … Deng, Y. B. (2013). Suppressive effects of dietary high fluorine on the intestinal development in broilers. *Biological Trace Element Research*, *156*, 153–165.

Mukhopadhyay, D., Srivastava, R., & Chattopadhyay, A. (2015). Sodium fluoride generates ROS and alters transcription of genes for xenobiotic metabolizing enzymes in adult zebrafish (Danio rerio) liver: Expression pattern of Nrf2/Keap1 (INrf2). *Toxicology Mechanisms and Methods*, *25*(5), 1–10.

NBI (Nile Basin Initiative) (2005).Nile basin national water quality monitoring baseline study report for Egypt, Nile Transboundary Environmental Action Project, Nile Basin Initiative.

Norman, H. O., & Arden, C. G. (1991). *Water fluoridation in primary preventive dentistry*, (3rd ed.,). Stamford: Appleton & Lange.

Oyagbemi, A. A., Omobowale, T. O., Asenuga, E. R., Adejumobi, A. O., Ajibade, T. O., Ige, T. M., … Yakubu, M. A. (2017). Sodium fluoride induces hypertension and cardiac complications through generation of reactive oxygen species and activation of nuclear factor kappa beta. *Environmental Toxicology*, *32*(4), 1089–1101.

Paget, G. E., & Barnes, J. M. (1964). Toxicity tests. In D. R. Laurence, & A. L. Bachrach (Eds.), *Evaluation of Drug Activities: Pharmacometrics*, (vol. 1, pp. 135–166). London. New York: Academic Press.

Parikh, H., Tripathi, C. B., Shah, P., Ghori, V., & Goyal, R. K. (2015). Investigation of the cardioprotective effects of Crataegus oxycantha and its molecular mechanism. *Current Research Cardiology*, *2*(4), 161–167.

Ponikvar, M. (2008). Exposure of humans to fluorine and its assessment. *Fluorine Health*, *7*, 487–549.

Rahman, K., & Lowe, G. M. (2006). Significance of garlic and its constituents in cancer and cardiovascular disease. *The Journal of Nutrition*, *136*, 736–740.

Ravichandran, B., Chattopadhyay, S., Gangopadhyay, P. K., & Saiyed, H. N. (2012). Evaluation of hematological changes in population exposed to fluoride. *Toxicological and Environmental Chemistry*, *94*(10), 2052–2056.

Reitman, S., & Frankel, S. A. (1957). A colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, *28*(1), 56–63.

Reitz, B., Isence, H., Strobach, H., Makdessi, S., & Jacob, R. (1993). Cardioprotective actions of wild garlic (Allium urisenum) in ischemia and reperfusion. *Molecular and Cellular Biochemistry*, *119*(1-2), 143–150.

Sarkar, P., Kumar, H., Rawat, M., Varshney, V. P., Goswami, T. K., Yadav, M. C., & Srivastava, S. K. (2006). Effect of administration of garlic extract and PGF2α on

hormonal changes and recovery in endometritis cows. *Asian-Australasian Journal of Animal Sciences*, *19*, 964–969.
Shanmugam, T., Selvaraj, M., & Poomalai, S. (2016). Epigallocatechin gallate potentially abrogates fluoride induced lung oxidative stress, inflammation via Nrf2/Keap1 signaling pathway in rats: An in-vivo and in-silico study. *International Immunopharmacology*, *39*, 128–139.
Shulman, J. D., & Well, L. M. (1997). Acute fluoride toxicity from ingesting home-use dental products in children, birth to 6 years of age. *Journal of Public Health Dentistry*, *57*, 150–158.
Song, G. H., Gao, J. P., Wang, C. F., Chen, C. Y., Yan, X. Y., Guo, M., … Huang, F. B. (2014). Sodium fluoride induces apoptosis in the kidney of rats through caspase-mediated pathways and DNA damage. *Journal of Physiology and Biochemistry*, *70*(3), 857–868.
Stawiarska-Pięta, B., Grzegorzak, N., Kuczera, K., Helis, A., Zalejska-Fiolka, J., Bielec, B., … Kubina, R. (2013). The effect of antioxidants on the morphological picture of thymus and heart of rats intoxicated with sodium fluoride. *Toxicology Letters*, *221*, S77.
Strubelt, O., Iven, H., & Younes, M. (1982). The pathophysiological profile of the acute cardiovascular toxicity of sodium fluoride. *Toxicology*, *24*, 313–323.
Susheela, A. K., & Kharb, P. (1990). Aortic calcification in chronic fluoride poisoning: Biochemical and electronmicroscope evidence. *Experimental and Molecular Pathology*, *53*, 72–80.
Švarc-Gajić, J., Stojanović, Z., Vasiljević, I., & Kecojević, I. (2013). Determination of fluorides in pharmaceutical products for oral hygiene. *Journal of Food and Drug Analysis*, *21*, 384–389.
Tuncel, E. (1984). The incidence of Moenckeberg calcifications in patients with endemic fluorosis. *Fluoride*, *17*(1), 4–8.
Upaganlawar, A., Gandhi, C., & Balaraman, R. (2009). Effect of green tea and vitamin E combination in isoproterenol induced myocardial infarction in rats. *Plant Foods for Human Nutrition*, *64*(1), 75–80.
Urdal, P., & Landaas, S. (1979). Macro creatine kinase BB in serum, and some data on its prevalence. *Clinical Chemistry*, *25*, 461–465.
Vasudevan, D. M., Sreekumari, S., & Kannnan, V. (2013). *Textbook of Biochemistry for Medical Students, Seventh edition*, (pp. 301–310). New Delhi: Jaypee Brothers Medical Publishers.
Wei, R., Luo, G., Sun, Z., Wang, S., & Wang, J. (2016). Chronic fluoride exposure-induced testicular toxicity is associated with inflammatory response in mice. *Chemosphere*, *153*, 419–425.
WHO (1993). *Guidelines for Drinking Water Quality*, (p. 45). Geneva: World Health Organization.
Yeh, Y.-Y., & Liu, L. (2001). Cholesterol-lowering effect of garlic extracts and organosulfur compounds: Human and animal studies. *The Journal of Nutrition*, *131*(3), 989–993.
Yildirim, S., Ekin, S., Huyut, Z., Oto, G., Comba, A., Uyar, H., … Cinarf, D. A. (2018). Effect of chronic exposure to sodium fluoride and 7,12- dimethylbenz [A] anthracene on some blood parameters and hepatic, renal, and cardiac histopathology in rats. *Fluoride*, *51*(93), 278–290.
Young, D.S. (2001). Effects of disease on Clinical Lab. Tests, 4th ed AACC.
Zheng, X., Sun, Y., Ke, L., Ouyang, W., & Zhang, Z. (2016). Molecular mechanism of brain impairment caused by drinking-acquired fluorosis and selenium intervention. *Environmental Toxicology and Pharmacolgy*, *43*, 134–139.

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