

PROTECTIVE EFFECT OF METHYL 3-O-METHYL GALLATE AGAINST SODIUM FLUORIDE-INDUCED OXIDATIVE STRESS IN RAT CARDIAC TISSUES

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SUMMARY: Methyl 3-O-methyl gallate (M3OMG), a naturally-occurring compound of limited distribution, can be readily synthesized from methyl gallate. In the present study, the protective effect of M3OMG of synthetic origin on fluoride-induced cardiotoxicity and oxidative stress in cardiac tissues of rats was evaluated. After intraperitoneal administration of M3OMG (10 and 20 mg/kg bw), or the positive control (ascorbic acid at 10 mg/kg bw), for seven consecutive days, rats were intoxicated by fluoride (F) with sodium fluoride (NaF) at 600 ppm in their drinking water for a further week. The level of thiobarbituric acid reactive substances (TBARS) and reduced glutathione (GSH) and the activities of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were examined in the cardiac homogenates. The results showed that F intoxication caused a significant abnormality in the levels of TBARS and GSH as well as in the activities of antioxidant enzymes in cardiac tissues. M3OMG at the dose of at 20 mg/kg bw significantly suppressed the NaF-induced enhanced level of TBARS. M3OMG also restored the NaF-induced abnormality in the activities of SOD and CAT and the level of GSH in cardiac tissues. Ascorbic acid (vitamin C) at 10 mg/kg bw also showed a comparable amelioration of the NaF-induced toxicity in the rat heart. Taking all the results together, M3OMG demonstrated a notable cardioprotective role against NaF-induced toxicity and oxidative stress in rat cardiac tissues.

Keywords: Cardiac stress; Fluoride in rats; Lipid peroxidation; Methyl-3-O-methyl gallate; Oxidative stress in rats.

INTRODUCTION

Fluorosis is a worldwide crucial health problem in at least 25 countries of the world.¹ It has recently been reported that chronic fluorosis causes severe metabolic, functional, and structural damage in mammalian cells. Moreover, the simultaneous consumption of fluoridated water and fluoride (F) supplements and the use of fluoridated toothpaste and F in infant formulas can cause marginal fluorosis, including dental fluorosis, before the age of six years.² F anions stimulate oxidizing agents that reversibly inhibit the enzymatic activity of at least 80 proteins.³ They have been shown to cause oxidative damage and lipid peroxidation, regulate redox homeostasis, and induce apoptosis in cells through alteration of gene expression.^{4,5} To date, the exact molecular mechanism of F toxicity is unclear, but several studies have shown that mitochondria are key targets of F toxicity in the cells.⁵ Hence, F abnormality is more evident in mitochondria-rich cells such as heart tissues. Recently, we have found that natural

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antioxidants such as quercetin can mitigate F-induced oxidative stress in the cardiac tissues of rats.⁶

Recent studies highlighted the isolation of an interesting antioxidant compound, methyl-3-O-methyl gallate (M3OMG, Figure 1), from *Peltiphyllum peltatum*.⁷ Comparative *in vitro* studies on the antioxidant/pro-oxidant activities of M3OMG and gallic acid revealed that M3OMG (unlike gallic acid) displayed antioxidant activities without overtly being a pro-oxidant.⁷ In continuation of our research, the present work was designed to determine the possible *in vivo* antioxidant effect of M3OMG against NaF-induced oxidative stress in the cardiac issues of rats. In order to secure sufficient amounts of M3OMG for testing, we have synthesized the compound in gram quantities.

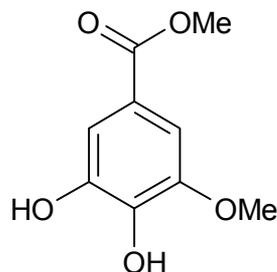


Figure 1. Chemical structure of methyl 3-O-methylgallate (M3OMG).

MATERIALS AND METHODS

Chemicals: Bovine serum albumin and a kit for protein measurement were purchased from Ziest Chem Co., (St Louis, MO, USA). All other chemicals used in present study were of analytical grade and were purchased from either Sigma Chemical Co., (St Louis, MO, USA) or Sigma-Aldrich Chemical Company (Dorset, UK).

Synthesis of M3OMG: Borax (80 g) and water (800 mL) were added to methyl gallate (10 g) in a reaction flask and the mixture stirred for 30 min. Dimethyl sulphate (30 mL) and NaOH (13 g in 50 mL of water) were then gently introduced dropwise (2.5 hr) from two sides of the reaction flask. After leaving the reaction mixture with stirring for 18 hr, conc. sulphuric acid (50 mL) was added. The mixture was further stirred for 1 hr and extracted (5-times) with chloroform (1 L). The combined chloroform extracts were washed with 500 mL of brine and dried over anhydrous sodium sulphate. Removal of the solvent under reduced pressure gave pure M3OMG (9.63 g).

Spectroscopic analysis: The JEOL 400 MHz instrument was used for recording ¹H NMR spectra of compounds with methanol-D₄ used as a solvent. The molecular mass of compounds was determined by using the VG BIO-Q mass spectrometer equipped with an ESI z-spray ion source. Samples dissolved in methanol were directly injected into the mass spectrometer operating in a negative ion mode. The mass spectrometer was operated with the following ESI-settings: capillary voltage 4.02 kV, cone voltage: 44V, source temperature 126°C, desolvation temperature, 28°C. For full scan MS analysis, the spectra were acquired in the range of 50–1000 m/hz. The prominent ion was selected for CID (collision induced dissociations) fragmentations, and the abundant ions were further subjected to MS analysis.

Animals: Male Wistar rats (200–250 g) obtained from Pasteur Institute of Iran (branches of Amol) were used for the study. They were kept in the University of Mazandaran (Babolsar, Iran) animal house facility and maintained at 25°C with a

12 hr light/12 hr dark cycle. They were fed a standard pellet rodent diet and allowed to acclimatize for 14 days prior to being used for the study.

Ethical statement: All experiments were conducted in accordance with the norms of the ethical committee of University of Mazandaran, Babolsar, following the strict guidelines of the Principles of Laboratory Animals Care (NIH Publication no. 85-23, revised 1996).

Experimental procedure: The rats were randomly divided into five separate groups containing 10 animals each. The first two groups were treated with 5% dimethyl sulfoxide vehicle, which served as normal (non-intoxicated group) and the control group. The control group also received for one week 600 ppm NaF in their drinking water. The next two groups received M3OMG (10 and 20 mg/kg bw) intraperitoneally for 7 days prior to NaF intoxication. Animals of the last group served as the positive control group, receiving vitamin C (10 mg/kg bw) intraperitoneally and intoxicated with NaF under the same experimental conditions.⁶

Preparation of homogenates: Following the treatment period, animals were anesthetized by intraperitoneal administration of ketamine (60 mg/kg bw) and xylazine (5 mg/kg bw). The heart was then removed and washed three times with isotonic saline. Homogenates of cardiac tissues were prepared in a solution of potassium dihydrogen phosphate buffer containing ethylenediaminetetraacetic acid. After centrifugation at 16000 g for 30 min, the supernatants were carefully collected and used for the biochemical analysis.

Biochemical analyses: The total protein content of the cardiac homogenate samples was measured by the Bradford method.⁸ The method of Ohkawa et al.⁹ was used for the determination of lipid peroxidation level in the tissues. In this method, the level of lipid peroxidation was assessed by quantifying thiobarbituric acid reactive substances (TBARS), commonly expressed as malondialdehyde (MDA) content. Superoxide dismutase (SOD) activity in the cardiac tissues was examined by the method of Flohe and Ötting.¹⁰ In this assay model, “the amount of enzyme required for suppression of chromogen formation by 50%” is taken as a unit of SOD activity. For determination of catalase (CAT) activity, the method of Aebi¹¹ was used with a unit of activity is expressed as “the amount of catalase that transforms 1 μ mol of hydrogen peroxide into water.” The method of Ellman¹² was used for the determination of reduced glutathione (GSH) levels in the cardiac tissues.

Statistical analysis: All data points are expressed as means \pm S.D. Differences between experimental group means were analyzed through a one-way analysis of variance followed by Duncan’s multiple range tests.

RESULTS AND DISCUSSION

The starting material for the synthesis of M3OMG was methyl gallate. The use of a protective group (borax) allowed methylation of the peripheral C-3 hydroxyl group to give 9.63g (89.5% yield) of M3OMG. In the ¹H NMR spectrum, the two methyl groups of M3OMG appear as singlets at 3.83 and 3.86 while H-2 and H-6 protons were shown as doublet ($J=2.05$ Hz) signals at 7.14 and 7.16. The structure of M3OMG was further confirmed by ESI-MS analysis, which in the negative ion mode revealed the expected [M-1]⁻ molecular ion at 197.3.⁷

The levels of TBARS in cardiac tissues of all treated rats are summarized in Figure 2. It was observed that excessive consumption of NaF significantly increased the TBARS level when compared with untreated control group. The results also showed that M3OMG mitigated the NaF-induced augmented TBARS production in a dose-dependent manner (Figure 2).

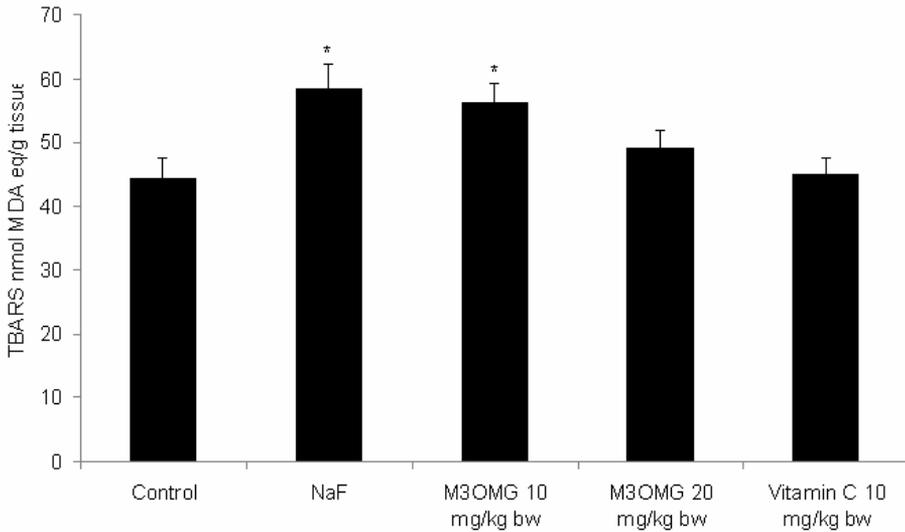


Figure 2. Effect of M3OMG on TBARS level in NaF-intoxicated rat heart. Data are mean \pm S.D. values ($n = 10$). *Significantly different versus untreated control group. $p < 0.05$ one-way ANOVA.

As shown in Figures 3 and 4, SOD and CAT activities were significantly suppressed when F intoxication was induced by administering 600 ppm NaF in the drinking water for one week.

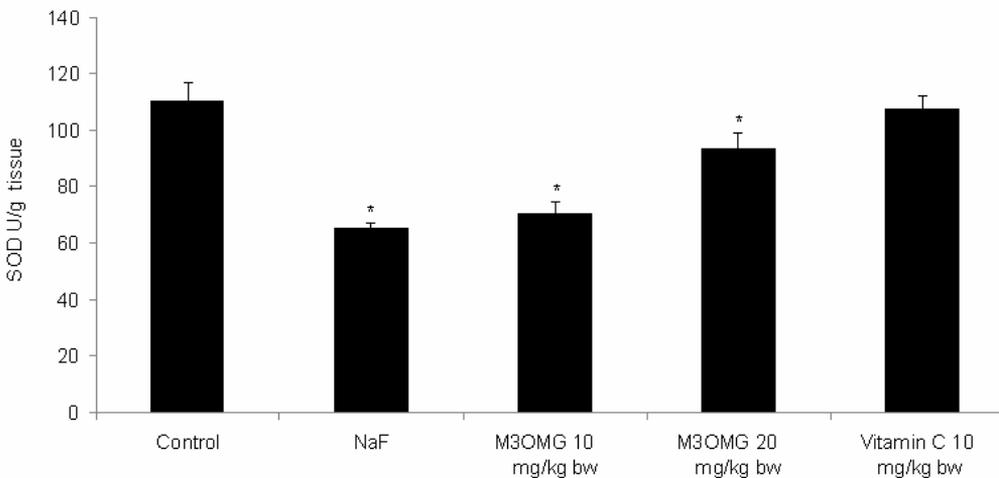


Figure 3. Effect of NaF intoxication on superoxide dismutase activity in the rat heart. The suppressive effect of M3OMG and ascorbic acid is shown. Data are mean \pm S.D. values ($n = 10$). *Significant differences with respect to control group. $p > 0.05$ one-way ANOVA.

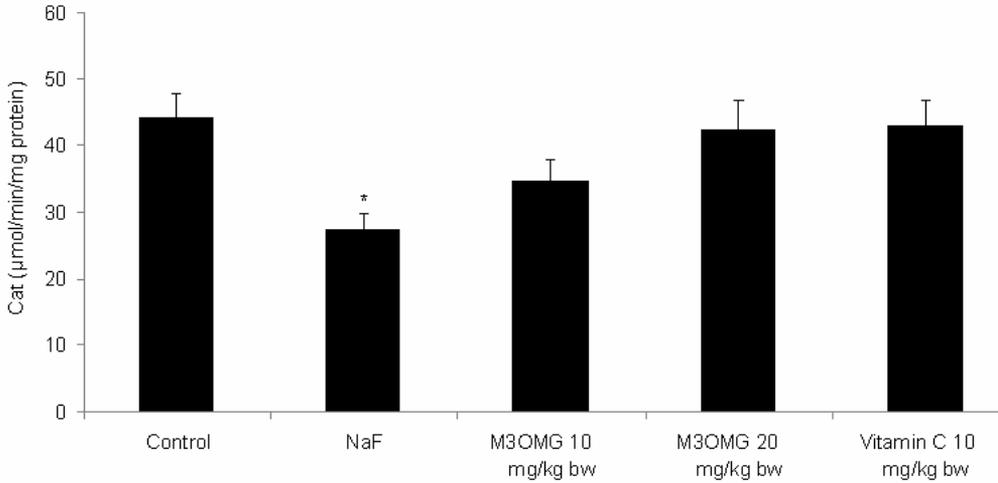


Figure 4. Effect of M3OMG treatment on catalase levels in NaF-intoxicated rat heart. Data are mean \pm S.D. values ($n = 10$). *Significantly different versus control group. $p < 0.05$ one-way ANOVA.

The NaF-mediated suppression of antioxidant enzymes activity was altered by intraperitoneal administration of M3OMG as well as by the positive control, vitamin C. Moreover, the observed restoration effect of the antioxidant enzymes SOD and CAST by M3OMG was also dose dependent.

The levels of reduced glutathione (GSH) in the cardiac tissues of the treated rats are shown in Figure 5.

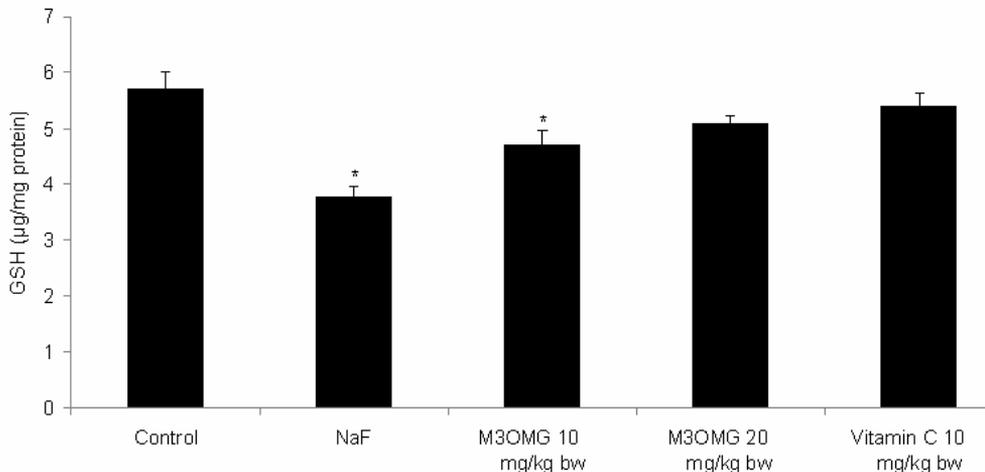


Figure 5. The effect of NaF on levels of reduced glutathione in the rat heart. Data are mean \pm S.D. values ($n = 10$). *Significantly different versus untreated control group. $p < 0.05$ one-way ANOVA.

It is clearly evident that treatment of rats with NaF for a week significantly reduced the level of GSH compared with the untreated control group ($p < 0.001$). It

was further observed that intraperitoneal administration of M3OMG prior to NaF intoxication restored the level of GSH in a dose-dependent manner.

To date, numerous scientific reports highlight the prominent role of reactive oxygen and nitrogenous species in the initiation and progression of different diseases conditions including the heart, and cardiovascular disorders.^{13,14} We have previously reported that F intoxication can lead to excessive production of reactive substances and affect the antioxidant-oxidant balance of hepatic tissue.¹⁵ It is also well known that excessive consumption of F causes cellular dysfunction by increasing the production of reactive substances such as nitric oxide, lipid peroxides, and reactive oxygen species.^{16,17} In the present study, we observed that excessive NaF consumption affected oxidant-antioxidant balance by increasing the levels of TBARS and decreasing the activities of SOD and CAT, and the levels of GSH in cardiac tissues. Our previous studies also established that natural products with antioxidant action can mitigate NaF-induced cardiotoxicity and abnormality in oxidative-antioxidant balance.⁶⁰

M3OMG is a low molecular weight antioxidant compound that has been isolated from very few species of plants.⁷ The compound is a mono *O*-methylated gallic acid methyl ester that still possesses the diorthohydroxyl functional moiety (Figure 1). As a result, the compound has been previously shown to have *in vitro* antioxidant activities, but, more interestingly, M3OMG was found to be a weaker prooxidant in cultured cells than gallic acid.⁷ The observation of these promising *in vitro* antioxidant effects prompted us to test the *in vivo* cardioprotective effect of M3OMG. In order to get gram quantities of M3OMG, we have employed a synthesis route, which provided the pure compound in a simple two-day reaction procedure. The identity of the compound was confirmed by means of NMR and mass spectroscopy.⁷

Our results confirm that F exposure causes oxidative damage as shown by the resulting reduced levels of antioxidant systems. It was also apparent that M3OMG mitigated the F-induced cardiotoxicity by modification of abnormality in the oxidant-antioxidant status measured by the changes in the level of TBARS, GSH, and activities of SOD and CAT in cardiac tissues of rats.

It is concluded that one-week intraperitoneal administration of M3OMG prior to F intoxication ameliorates oxidative stress in rat cardiac tissues. The results provide direct *in vivo* evidence for the potential use of M3OMG as a cardioprotective antioxidant agent. Future studies should investigate the possible mechanisms of cardioprotective effect of M3OMG and its further therapeutic, toxicological, and pharmacokinetic profiles.

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REFERENCES

- 1 Dai SF, Li WW, Tang YG, Zhang Y, Feng P. The sources, pathway, and preventive measures for fluorosis in Zhijin County, Guizhou, China. *Appl Geochem* 2007;22(5):1017–24.

- 2 Buzalaf MA, Levy SM. Fluoride intake of children: considerations for dental caries and dental fluorosis. *Monogr Oral Sci* 2011;22:1-19.
- 3 García-Montalvo EA, Reyes-Pérez H, Del Razo LM. Fluoride exposure impairs glucose tolerance via decreased insulin expression and oxidative stress. *Toxicology* 2009;19;263(2-3):75-83.
- 4 Basha PM, Sujitha NS. Combined influence of intermittent exercise and temperature stress on the modulation of fluoride toxicity. *Biol Trace Elem Res* 2012;148(1):69-75.
- 5 Barbier O, Arreola-Mendoza L, Del Razo LM. Molecular mechanisms of fluoride toxicity. *Chem Biol Interact* 2010;188(2):319-33.
- 6 Nabavi SF, Nabavi SM, Mirzaei M, Moghaddam AH. Protective effect of quercetin against sodium fluoride induced oxidative stress in rat's heart. *Food Funct* 2012; 3(4):437-41.
- 7 Habtemariam S. Methyl-3-O-methyl gallate and gallic acid from the leaves of *Peltiphyllum peltatum*: isolation and comparative antioxidant, prooxidant, and cytotoxic effects in neuronal cells. *J Med Food* 2011;14:1412-8.
- 8 Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248-54.
- 9 Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- 10 Flohé L, Ötting F. Superoxide dismutase assays. *Methods Enzymol* 1984;105:93-104.
- 11 Aebi H. Catalase *in vitro*. *Methods Enzymol* 1984;105:121-6.
- 12 Ellman GL. Tissue sulphhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
- 13 Al Ghouleh I, Khoo NK, Knaus UG, Griendling KK, Touyz RM, Thannickal VJ, et al. Oxidases and peroxidases in cardiovascular and lung disease: new concepts in reactive oxygen species signaling. *Free Radic Biol Med* 2011;51(7):1271-88.
- 14 Chen K, Keaney JF Jr. Evolving concepts of oxidative stress and reactive oxygen species in cardiovascular disease. *Curr Atheroscler Rep* 2012;14(5):476-83.
- 15 Nabavi SM, Nabavi SF, Eslami S, Moghaddam AH. *In vivo* protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. *Food Chem* 2012;132:931-5.
- 16 Nabavi SF, Moghaddam AH, Eslami S, Nabavi SM. Protective effects of curcumin against sodium fluoride-induced toxicity in rat kidneys. *Biol Trace Elem Res* 2012;145:369-74.
- 17 Grucka-Mamczar E, Birkner E, Błaszczuk I, Kasperczyk S, Wielkoszyński T, Świętochowska E, et al. The influence of sodium fluoride and antioxidants on the concentration of malondialdehyde in rat blood plasma. *Fluoride* 2009;42(2):101–4.