



## Effect of silymarin on sodium fluoride-induced toxicity and oxidative stress in rat cardiac tissues

SEYED M. NABAVI<sup>1,2</sup>, SEYED F. NABAVI<sup>1,2</sup>, AKBAR H. MOGHADDAM<sup>3</sup>,  
WILLIAM N. SETZER<sup>4</sup> and MORTEZA MIRZAEI<sup>1</sup>

<sup>1</sup>Applied Biotechnology Research Center, Baqiyatallah University of Medical Sciences, Tehran, Sheikh Bahaei St, 19395-5487, Iran

<sup>2</sup>National Elites Foundation of Iran, Tehran, Shirkooh St, 14174, Iran

<sup>3</sup>Department of Biology, Faculty of Sciences, University of Mazandaran, Babolsar, Pasdaran St, 47415, Iran

<sup>4</sup>Department of Chemistry, University of Alabama in Huntsville, Huntsville, Alabama, 35899, USA

*Manuscript received on January 11, 2012; accepted for publication on June 28, 2012*

### ABSTRACT

This study aim to evaluate the protective effect of silymarin on sodium fluoride-induced oxidative stress in rat cardiac tissues. Animals were pretreated with silymarin at 20 and 10 mg/kg prior to sodium fluoride consumption (600 ppm through drinking water). Vitamin C at 10 mg/kg was used as standard antioxidant. There was a significant increase in thiobarbituric acid reactive substances level ( $59.36 \pm 2.19$  nmol MDA eq/g tissue) along with a decrease in antioxidant enzymes activity ( $64.27 \pm 1.98$  U/g tissue for superoxide dismutase activity and  $29.17 \pm 1.01$   $\mu\text{mol}/\text{min}/\text{mg}$  protein for catalase activity) and reduced glutathione level ( $3.8 \pm 0.15$   $\mu\text{g}/\text{mg}$  protein) in the tissues homogenates of the sodium fluoride-intoxicated rats. Silymarin administration to animals before sodium fluoride consumption modified the levels of biochemical parameters.

**Key words:** catalase, glutathione, sodium fluoride, superoxide dismutase, silymarin, TBARS.

### INTRODUCTION

Fluoride in water sources is known to induce both useful and harmful influence on human body. In intoxication, fluoride induces skeletal and dental fluorosis which causes damage to major tissues of the body such as the heart tissues (Sinha et al. 2008). Fluoride accumulation in soft tissues causes oxidative stress through inhibition of different enzymatic systems and increased generation of free radicals (Nabavi et al. 2012c). Fluoride-induced oxidative stress plays an important role

in progression of a variety of cardiac disorders such as cardiac failure and ischemia (Sinha et al. 2008). Therefore, oxidant-antioxidant balance is an important mechanism in mitigation of the oxidative stress in cardiac tissues. Sinha et al. (2008) have shown that fluoride consumption causes myocardium injuries and dysfunction. Also, Nabavi et al. (2012c) have reported that fluoride increases oxidative stress through abnormal biochemical parameters in different tissues of rats. The free radical-induced oxidative stress is well known as an important mechanism of fluoride intoxication (Nabavi et al. 2012c).

Correspondence to: Seyed Mohammad Nabavi  
E-mail: nabavi208@gmail.com

Reactive oxygen species have an important role in cardiac failures. Knowing that reactive oxygen species play a crucial role in fluoride-induced cardiotoxicity and oxidative stress, studies have been carried out on the cardioprotective action of antioxidants against fluoride-induced toxicity in cardiac tissues (Sinha et al. 2008). Recent reports have determined the mitigative action of herbal antioxidants in some cardiac failures caused by free radicals and other reactive species (Nabavi et al. 2012c). Previously, the authors reported that natural antioxidants mitigated the oxidative injuries of fluoride in the cardiac tissues of rat (Nabavi et al. 2012b).

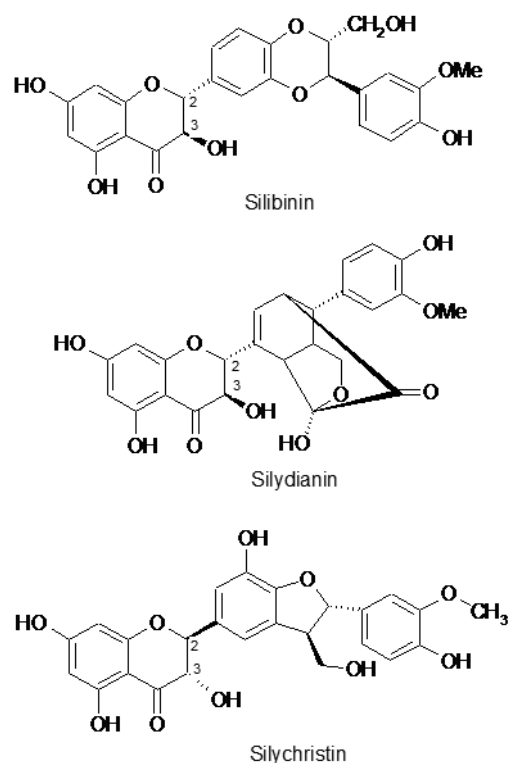
Silymarin (Figure 1) is a mixture of three benzopyranone compounds and well known as a herbal antioxidant constituent of *Silybum marianum* (milk thistle). Silybin (a), silydianin (b), and silychristin (c) (Figure 1) are major components of silymarin (Rašković et al. 2011). Its useful effects have been correlated to the antioxidant, antiproliferative and antiinflammatory actions on the basis of the specific signaling pathway regulation, transcription factor and gene expression (Rao and Viswanath 2007).

The purpose of the present work is to determine the ameliorative action of silymarin through its antioxidant effect in the cardiac tissues of rats exposed to sodium fluoride.

## MATERIALS AND METHODS

### CHEMICALS

Bovine serum albumin and a kit for protein measurement were purchased from ZiestChem Company (Tehran, Iran). Silymarin, 5,5'-dithiobis (2-nitrobenzoic acid), glacial acetic acid, heparin, nitro blue tetrazolium chloride, potassium dihydrogen phosphate, reduced glutathione, sodium dihydrogen phosphate, sodium fluoride, trichloroacetic acid, thiobarbituric acid, hydrogen peroxide, vitamin C, ferric chloride, and hydroxylamine hydrochloride were purchased from Sigma-Aldrich Chemical



**Figure 1** - Components of silymarin: (a) silybin, (b) silydianin, and (c) silychristin.

Company, (St Louis, MO, USA). Ketamine and xylazine hydrochloride were purchased from Alfason-Woerden Company (Amsterdam, Holland). Other chemical reagents and solvents were of analytical grade or better.

### ANIMALS

Male Wistar rats were purchased from Pasteur Institute of Iran, Amol, and kept in an animal house of the Department of Biology, College of Sciences, University of Mazandaran, weighing between 200-250 g. The animals were allowed access to pellet diet and water *ad libitum* for two weeks prior to the experiments, for their acclimatization. All of the animals were kept in ventilated cages at 28-30°C, and 12/12 h light/dark cycles. All procedures were performed according to the norm of the ethical committee of University of Mazandaran Institutional Animal Care and Use Committee (Approval number: No.S-2009 UMZ).

#### TREATMENT PROCEDURE

Rats were divided into the 5 groups containing 10 rats per groups. Animals of group I received the vehicle only (1 mg/kg/day) intraperitoneally for 1 week and served as normal. Silymarin at 10 and 20 mg/kg/day was administrated intraperitoneally to animals of groups II and III for 1 week prior to sodium fluoride intoxication (600 ppm) for next week (treated groups). Vitamin C (10 mg/kg/day), as a standard antioxidant, was administrated intraperitoneally to animals of group IV prior to sodium fluoride intoxication and served as positive controls. Finally, group V were pretreated with vehicle for same period prior to sodium fluoride intoxication (600 ppm) and served as toxin control.

#### ANIMAL ANESTHESIA

Rats were anesthetized with mixture of ketamine (60 mg/kg) and xylazine (5 mg/kg) and killed 24 hours after the last application. Animal heart was removed and washed three times by normal saline for complete blood removal.

#### HOMOGENATE PREPARATION

The whole heart tissues were weighed and homogenized in chilled potassium chloride (1.15%). The homogenates were centrifuged at 6,000 g for 15 min at 4°C. The supernatant was used for biochemical analysis.

#### PROTEIN DETERMINATION

The protein concentration was evaluated according to the method of Bradford (1976) using bovine serum albumin as the standard.

### BIOCHEMICAL ANALYSIS

#### ESTIMATION OF LIPID PEROXIDATION ASSAY

TBARS in heart tissues was evaluated by reaction with thiobarbituric acid (Shahidi et al. 2002). Briefly, reaction mixture (1.0 mL) was contained 0.1 M of

phosphate buffer (0.58 mL; pH 7.4), tissue homogenate (0.2 mL), 100 mM of ascorbic acid (0.2 mL) and 100 mM of ferric chloride (0.02 mL). After incubation at 37°C for 60 min, reaction was stopped by addition of 10% of trichloroacetic acid (1.0 mL). Then, 1.0 mL of thiobarbituric acid (0.67%) was added to the reaction mixture and placed in boiling water bath for 20 min. The amount of thiobarbituric acid reactive substances formed was examined by recording the absorbance of reaction mixture at 535 nm using spectrophotometer against a reagent blank.

#### DETERMINATION OF SUPEROXIDE DISMUTASE ACTIVITY

Superoxide dismutase activity was evaluated through the method of Nabavi et al. (2012a). Reaction mixtures included 1 mL of sodium carbonate (50 mM), 0.4 mL of nitroblue tetrazolium (25  $\mu$ M) and 0.2 mL of hydroxylamine hydrochloride (0.1 mM). The homogenates (0.1 mL, 1:10, w/v) were added to the reaction mixtures. Finally, the absorbance of the reaction mixture was recorded at 560 nm.

#### DETERMINATION OF CATALASE ACTIVITY

Catalase activity was evaluated according to the method of Nabavi et al. (2012c). Briefly, a reaction mixture (3 mL) containing 50 mM of phosphate buffer (2.5 mL; pH 5.0), 5.9 mM of H<sub>2</sub>O<sub>2</sub> (0.4 mL) and heart homogenate (0.1 mL) was incubated for one min and absorbance of the sample was recorded at 240 nm.

#### DETERMINATION OF REDUCED GLUTATHIONE ACTIVITY

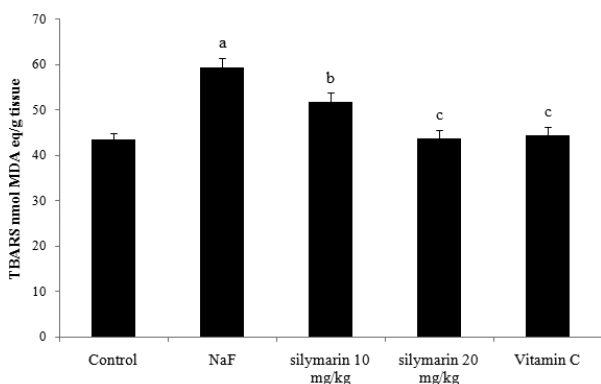
Reduced glutathione level was evaluated through the method of Ellman (1959). The homogenate sample (720  $\mu$ L) was diluted and then trichloroacetic acid (5%) was added to the reaction mixture for precipitation of protein content in tissue homogenates. Reaction was centrifuged at 10,000 g for 5 min and then the supernatant was taken. Ellman's reagent [5,5'-dithiobis (2-nitrobenzoic acid) solution] was added to the sample. Finally, the absorbance of the sample was recorded at 417 nm.

## STATISTICAL ANALYSIS

The values are presented as means  $\pm$  S.D. Differences between group means were estimated using a one-way analysis of variance followed by Duncan's multiple range tests. Results were considered statistically significant when  $p < 0.05$ .

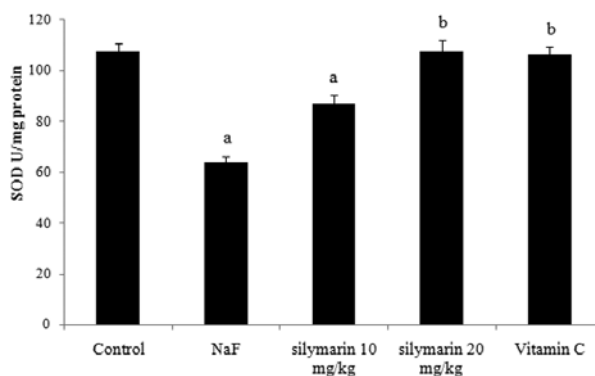
## RESULTS AND DISCUSSION

Figure 2 contain TBARS level (lipid peroxidation) of rat hearts. The results demonstrate that one week of fluoride intoxication increases TBARS level in rat heart ( $59.36 \pm 2.19$  nmol MDA eq/g tissue) in comparison with normal rat ( $43.51 \pm 1.47$  nmol MDA eq /g tissue). Also we found that pretreatment with Silymarin and vitamin C inhibit fluoride-induced lipid peroxidation levels ( $51.83 \pm 2$  nmol MDA eq /g tissue for 10 mg/kg of silymarin;  $43.7 \pm 1.99$  nmol MDA eq /g tissue for 20 mg/kg of silymarin; and  $44.52 \pm 1.73$  nmol MDA eq /g tissue for vitamin C).



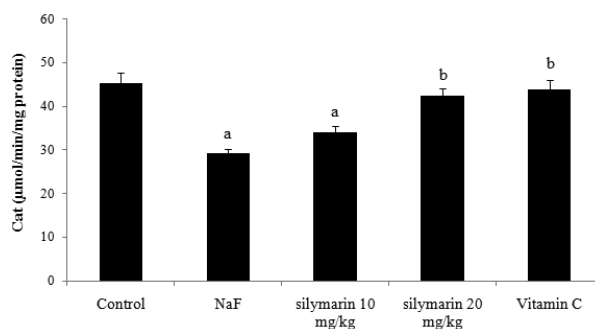
**Figure 2** - Effect of silymarin and vitamin C on TBARS level in heart's of sodium fluoride intoxicated rat. <sup>a</sup> $p < 0.001$  versus normal rats. <sup>b</sup> $p < 0.01$  versus normal group. <sup>c</sup> $p > 0.05$  versus normal group.

Results of superoxide dismutase activity have been summarized in Figure 3. We observed that pretreatment with silymarin and vitamin C diminished fluoride-induced abnormality in superoxide dismutase activity level of rat heart ( $86.88 \pm 3.66$  U/mg protein for 10 mg/kg,  $107.74 \pm 4.4$  U/mg protein for 20 mg/kg and  $106.25 \pm 3.01$  U/mg protein for vitamin C Vs.  $64.27 \pm 1.98$  U/mg protein for fluoride intoxicated rat).



**Figure 3** - Effect of sodium fluoride intoxication on superoxide dismutase activity in the rat's heart. <sup>a</sup> $p < 0.001$  versus normal rats. <sup>b</sup> $p > 0.05$  versus normal group.

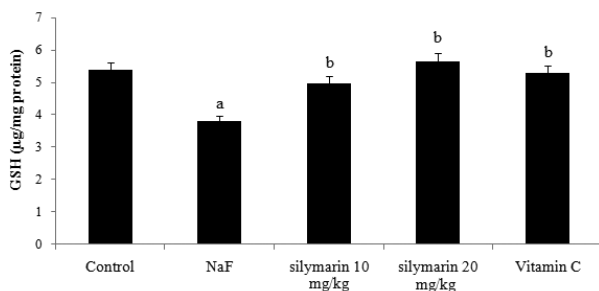
Also, we observed a similar pattern in catalase activity (Figure 4). Fluoride intoxication decreased enzyme activity ( $29.17 \pm 1.01$   $\mu$ mol/min/mg protein) compared with the control group ( $45.36 \pm 2.27$   $\mu$ mol/min/mg protein). Pretreatment with silymarin and vitamin C preserved catalase activity in comparison to fluoride-intoxicated rat ( $34.12 \pm 1.3$   $\mu$ mol/min/mg protein for 10 mg/kg;  $42.5 \pm 1.6$   $\mu$ mol/min/mg protein for 20 mg/kg for silymarin; and  $43.93 \pm 2.18$  U/mg protein for vitamin C).



**Figure 4** - Effect treatment on catalase levels in sodium fluoride intoxicated rat. <sup>a</sup> $p < 0.001$  versus normal rats. <sup>b</sup> $p > 0.05$  versus normal group.

The data of reduced glutathione levels in heart homogenates are summarized in Figure 5. The results show that fluoride exposure for one week significantly decreased reduced glutathione levels ( $3.8 \pm 0.15$   $\mu$ g/mg protein). Silymarin administration evidently protects against fluoride-induced decreases in the level of reduced glutathione ( $4.98 \pm 0.22$   $\mu$ g/mg protein for 10 mg/kg and  $5.65 \pm$

0.26  $\mu\text{g}/\text{mg}$  protein for 20 mg/kg). Treatment with Vitamin C showed mitigating activity similar to silymarin ( $5.3 \pm 0.21 \mu\text{g}/\text{mg}$  protein).



**Figure 5** - Protective effect of silymarin on sodium fluoride induced abnormality in reduced glutathione level in the heart homogenate of rat. <sup>a</sup> $p < 0.001$  versus normal rats. <sup>b</sup> $p > 0.05$  versus normal group.

It is well known that an increase in reactive oxygen species formation leads to oxidative stress, which has an important role in pathogenesis of different illnesses including cardiovascular, inflammation etc.

Among different types of reactive oxygen species, hydrogen peroxide plays a crucial role in oxidative damage. Furthermore, hydrogen peroxide, thiobarbituric acid reactive substances and the conjugated diene formation in the cardiac tissues were increased in acute heart failure, while antioxidant enzymes such as superoxide dismutase and catalase protected from these abnormalities.

It has been reported that fluoride consumption leads to excessive formation of free radical and other reactive substances and diminishes the activity of antioxidant enzymes (Nabavi et al. 2012c). Liu et al. (2003) demonstrated that fluoride consumption increased production of lipid peroxides, reactive nitrogen and oxygen species, leading to cellular damage, which affects the cellular systems. Fluoride caused oxidative stress in the cells by biphasic mechanisms; it acts as a free radical generator as well as an inhibitor of endogenous antioxidant enzyme systems (Ranjan et al. 2009). Sodium fluoride intoxication increases the level of lipid peroxidation along with a decrease in antioxidant enzymatic activities (superoxide

dismutase and catalase) and in the level of reduced glutathione in rat cardiac tissues (Sinha et al. 2008). Previously, it has been reported that some natural products with antioxidant ability have mitigative action (Nabavi et al. 2012c).

Natural antioxidants such as phenols and flavonoids show protective actions in different models of toxin-induced oxidative stress in cardiac tissues. Silymarin is a mixture of flavonoid components, and this natural antioxidant is isolated from *Silybum marianum* L. Gaertn (Milk thistle). Silymarin is composed primarily of three polyphenolic benzopyranone components, silybin, silydianin, and silychristin (Lee and Liu 2003). Silymarin has anti-inflammatory, anti-oxidative and anti-carcinogenic activities and so it has been tested in various in-vitro and in-vivo models of oxidative stress for protection of oxidative injuries in different tissues (Chtourou et al. 2010).

Previously, the protective roles of silymarin in doxorubicin-induced cardiotoxicity (Rašković et al. 2011) and ischemia-reperfusion-induced myocardial infarction-induced cytotoxicity has been reported (Rao and Viswanath 2007).

In this paper, we examine fluoride-induced oxidative injuries in cardiac tissues, which increase the production of reactive oxygen species. Furthermore, we examine the ameliorative actions of silymarin, a natural antioxidant, on fluoride-induced oxidative stress in rat cardiac tissues. In our present study, TBARS and reduced glutathione levels and activities of antioxidant enzymes (i.e., superoxide dismutase and catalase in rat cardiac tissues) were examined to evaluate the protective action. Antioxidants play a crucial role in mitigation and/or protection against oxidative stress induced by sodium fluoride through directly scavenging and/or inhibiting reactive oxygen species. Combining results of present study show that intraperitoneally administration of silymarin before sodium fluoride consumption prevents oxidative stress in rat cardiac tissues due to the



antioxidant actions of this compound. According to the results of the present work, silymarin-rich foods can protect cardiac tissues from fluoride-induced oxidative stress. Future studies are needed for a better understanding of preventive and/or protective action mechanisms. These results can benefit future studies on silymarin for its applications in drug discovery and development.

#### ACKNOWLEDGMENTS

The authors acknowledge the financial support of National Elite's Foundation of Iran (Tehran, Iran) for this study. This paper is dedicated to Seyed Maryam Nabavi and to the memory of Seyed Ali Asghar Nabavi.

#### RESUMO

Este estudo objetiva avaliar o efeito protetor da silimarina em fluoreto de sódio induzida por estresse oxidativo em tecido cardíaco de ratos. Os animais foram pré-tratados com silimarina a 20 e 10 mg/kg antes do consumo de fluoreto de sódio (600 ppm através da água de beber). A vitamina C a 10 mg/kg foi utilizada como antioxidante padrão. Houve um aumento significativo no nível de substâncias tiobarbitúrico reativo de ácido ( $59,36 \pm 2,19$  nmol MDA eq/g tecido), juntamente com uma diminuição da atividade de enzimas antioxidantes ( $64,27 \pm 1,98$  U/g tecido para a atividade de superóxido dismutase e  $29,7 \pm 1,01$  mmol/min/mg de proteína para a atividade da catalase) e nível de glutatona reduzida ( $3,8 \pm 0,15$  mg/mg de proteína) nos homogeneizados de tecidos dos fluoreto de sódio-intoxicados ratos. Administração de silimarina a animais, antes do consumo de fluoreto de sódio modificou os níveis de parâmetros bioquímicos.

**Palavras-chave:** catalase, glutatona, fluoreto de sódio, superóxido dismutase, silimarina, TBARS.

#### REFERENCES

- BRADFORD MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248-254.
- CHTOUROU Y, FETOUI H, SEFI M, TRABELSI K, BARKALLH M, BOUDAWARA T, KALLEL H AND ZEGHAL N. 2010. Silymarin, a natural antioxidant, protects cerebral cortex against manganese-induced neurotoxicity in adult rats. *Biomaterials* 23(6): 985-996.
- ELLMAN GL. 1959. Tissue sulphhydryl group. *Arch Biochem Biophys* 82: 70-77.
- LEE DY AND LIU Y. 2003. Molecular structure and stereochemistry of silybin A, silybin B, isosilybin A, and isosilybin B, Isolated from *Silybum marianum* (milk thistle). *J Nat Prod* 66: 1171-1174.
- LIU G, CHAI C AND CUI L. 2003. Fluoride causing abnormally elevated serum nitric oxide levels in chicks. *Environ Toxicol Pharmacol* 13: 199-204.
- NABAVI SF, NABAVI SM, ABOLHASANI F, MOGHADDAM AH AND ESLAMI S. 2012a. Cytoprotective effects of curcumin on sodium fluoride-induced intoxication in rat erythrocytes. *Bull Environ Cont Toxicol* 88: 486-490.
- NABAVI SF, NABAVI SM, MIRZAEI M AND MOGHADDAM AH. 2012b. Protective effect of quercetin against sodium fluoride induced oxidative stress in rat's heart. *Food Funct* 3(4): 437-441.
- NABAVI SM, NABAVI SF, ESLAMI S AND MOGHADDAM AH. 2012c. In vivo protective effects of quercetin against sodium fluoride-induced oxidative stress in the hepatic tissue. *Food Chem* 132: 931-935.
- RANJAN R, SWARUP D AND PATRA RC. 2009. Oxidative stress indices in erythrocytes, liver, and kidneys of fluoride-exposed rabbits. *Fluoride* 42(2): 88-93.
- RAO PR AND VISWANATH RK. 2007. Cardioprotective activity of silymarin in ischemia-reperfusion-induced myocardial infarction in albino rats. *Exp Clin Cardiol* 12(4): 179-187.
- RAŠKOVIĆ A, STILINOVIC N, KOLAROVIC J, VASOVIC V, VUKMIROVIC S AND MIKOV M. 2011. The protective effects of silymarin against doxorubicin-induced cardiotoxicity and hepatotoxicity in rats. *Molecules* 16(10): 8601-8613.
- SHAHIDI F, KAMIL J, JEON YJ AND KIM SK. 2002. Antioxidant role of Chitosan in a cooked cod (*Gadus morhua*) model system. *J Food Lipids* 9(1): 57-64.
- SINHAM, MANNA P AND SIL PC. 2008. Terminalia arjuna protects mouse hearts against sodium fluoride-induced oxidative stress. *J Med Food* 11(4): 733-740.