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Neuroprotective effects of silymarin on sodium fluoride-induced oxidative stress

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ABSTRACT

Silymarin is a well-known potent antioxidant agent. Numerous reports highlighted that antioxidant consumption can mitigate sodium fluoride induced neuronal damage. The present study aimed to examine the ameliorative potential of silymarin on sodium fluoride-induced oxidative stress using the rat brain as model. Silymarin (10 and 20 mg/kg) and vitamin C (10 mg/kg) were intraperitoneally administrated for seven days followed by one week of sodium fluoride (600 ppm) treatment through drinking water. Antioxidant enzyme activities – superoxide dismutase and catalase – the levels of reduced glutathione and lipid peroxidation were evaluated in brain homogenates. The levels of lipid peroxidation were significantly increased in the sodium fluoride treated group (42.0 ± 2.1 nmol MDA eq/g tissue) compared to the control group (36.0 ± 1.1 nmol MDA eq/g tissue). Silymarin at 20 mg/kg showed significant a reduction in the levels of lipid peroxidation (36.0 ± 1.2 nmol MDA eq/g tissue). Treatment of rats with sodium fluoride significantly reduced the activities of the antioxidant enzymes and the levels of reduced glutathione in brain homogenates. Pre-treatment with silymarin prevented the deleterious effects of sodium fluoride intoxication in a dose dependent response. In conclusion, sodium fluoride induced oxidative stress in rat brain which can be prevented with silymarin administration.

1. Introduction

Fluorinated compounds such as sodium fluoride, sodium fluorosilicate and cryolite (a fluoride-containing mineral) are used in various insecticide formulations and wood preservatives [1]. Consequently, these compounds and their derivates are ubiquitous in soil and water sources. Fluorinated compounds are also indirectly accumulated in foods such as tea through exposure of environmental pollutants, mainly pesticides [2]. Fluoride exposure to high doses causes intoxication in liver, heart, kidney, brain, erythrocytes, etc. [1,3–5]. The main compounds responsible of intoxications are sodium fluoride, hydrogen fluoride, sodium fluorosilicate and fluosilicic acid [6].

Fluoride anion is the most electronegative element in nature. Its intoxication leads to fluorosis, a common disorder in emerging countries, where oftentimes human's main drinking water source is contaminated with fluoride [7]. Fluoride easily distributes in the body through blood circulation, crosses the cellular membrane and its subsequent accumulation leads to cellular damage [2]. Neurodegeneration can occur when fluoride penetrates the brain through the blood stream [7]. In a fatal stage, fluoride accumulation leads to alterations in growth, cell differentiation, and subcellular organization in brain cells. In the affected area, neurological disorders such as partial paralysis of arms and legs, headache, visual disturbances, and mental retardation have been reported [6]. The free radical production is known to be one of the most important mechanism of fluoride toxicity [8].

Free radicals play a crucial role in various neurological symptoms such as neurodegenerative disorders [3]. There are several reports evidencing the neuroprotective effects of antioxidant agents against fluoride-induced toxicity in neural systems [6]. Recent studies have evaluated the protective effects of herbal constituents such as curcumin and quercetin as antioxidant agents against neurological diseases induced by reactive species [8]. It has been also reported that the antioxidant activity of plant constituents is mainly related to its natural compounds, such as flavonoids and phenolic acids [3].

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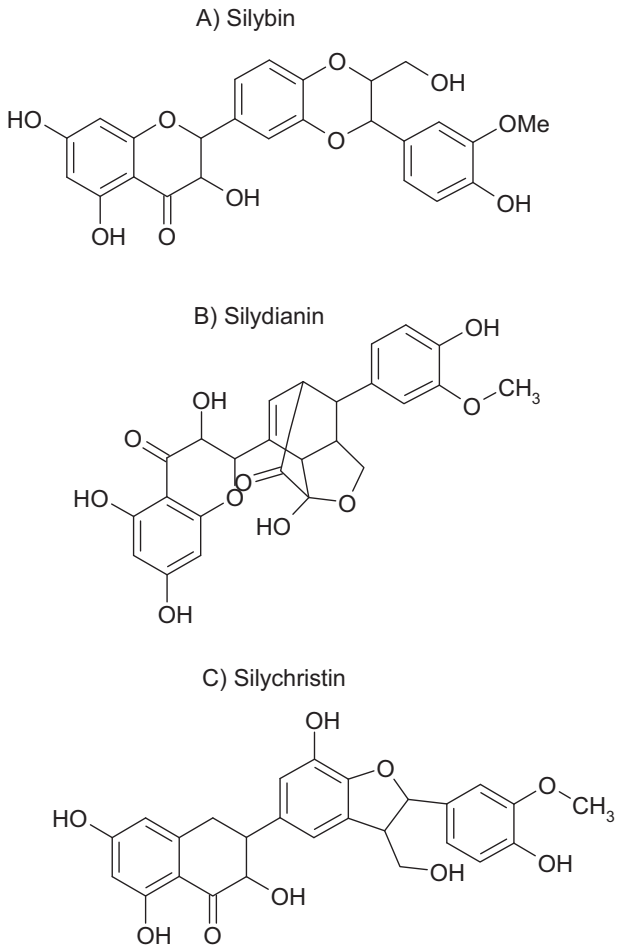

Fig. 1. Components of silymarin: (a) silybin, (b) silydianin, and (c) silychristin.

Silymarin (Fig. 1) is isolated from the fruits and seed of the plant *Silybum marianum* L. Gaertn (milk thistle) [9]. Milk thistle belongs to the family of Asteraceae and is indigenous of the Mediterranean area and southwest Europe [10]. The natural product of this plant is classified as a benzopyranone compound containing polyphenols such as silybin (a), silydianin (b) and silychristin (c) [9,11]. Milk thistle extract has centuries-old history of use in folk medicine to treat a variety of illnesses including jaundice, gallstones, hemorrhage, bronchitis or varicose veins. In recent times, it is more popular for the prevention and/or treatment of various liver disorders such as viral hepatitis and fatty liver [12,13]. Its beneficial effects have been attributed to the antioxidant, anti-proliferative and anti-inflammatory effects based on the regulation of specific signaling pathways, transcription factors and gene expression. Although it has been evidenced the potential benefits of silymarin, the protective actions towards the neural systems as well as its mechanisms of action need to be examined [14]. In the present study, we have examined whether silymarin administration induced any protective effect on the antioxidant parameters of the rat brain after intoxication with sodium fluoride.

2. Results

Fig. 2 shows the TBARS levels in rat neural tissues of the control, sodium fluoride, silymarin (10 and 20 mg/kg), and vitamin C (10 mg/kg) treated rats intoxicated with sodium fluoride. TBARS levels in the brain of sodium fluoride intoxicated rats were significantly increased when compared to the control group.

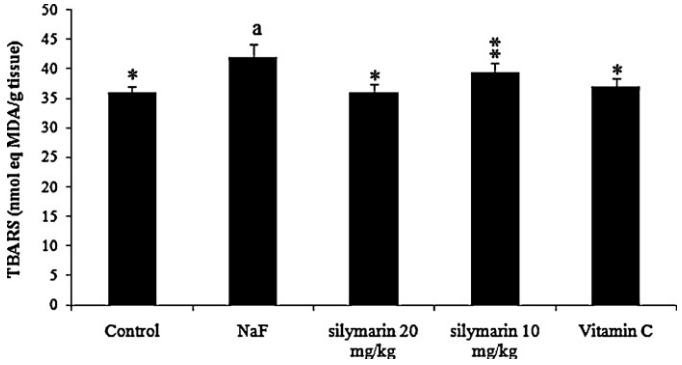

Fig. 2. TBARS levels in rat brain homogenates. Data are mean ± S.D. ($n = 10$). $^{a}P < 0.01$ versus control group. $^{*}P < 0.01$ versus NaF. $^{**}P > 0.05$ versus NaF.

Administration of silymarin at both concentrations and vitamin C reduced TBARS levels in neural tissues to values similar to the control group ($P < 0.05$).

Superoxide dismutase activity significantly decreased in rat neural tissues after sodium fluoride consumption respect to the control group (Fig. 3). The superoxide dismutase activity was completely normalized in the group treated with silymarin at 20 mg/kg but not in the group treated with silymarin at 10 mg/kg. The vitamin C treated group presented higher activities than the sodium fluoride group but this activity was lower than the control and silymarin 20 mg/kg groups ($P < 0.01$ versus control).

Catalase activity in homogenates of the rat neural tissues is reported in Fig. 4. Catalase activity in the neural tissues of sodium fluoride exposed rats was significantly lower than in the control group. Administration of silymarin prior to sodium fluoride intoxication through drinking water recovered the catalase activity in a dose dependent manner. Vitamin C treatment also recovered catalase activity.

Fig. 5 reports the levels of reduced glutathione in rat brain homogenates. Sodium fluoride consumption induced a significant decrease in the reduced glutathione when compared with the control group. Silymarin administration at high concentration prior to sodium fluoride exposure recovered the levels of reduced glutathione in rat neural tissues whereas neither the low concentration nor the vitamin C reported any significant change.

3. Discussion

The present study showed that sodium fluoride consumption induced significant changes in the antioxidant system and oxidative damage in rat brain. The administration of silymarin prior to sodium fluoride consumption modified the antioxidant response through mitigating the alterations in the analyzed biochemical parameters. Free radicals and other reactive species are continuously produced by the aerobic metabolism of living cells [3]. In addition, ultra-violet and ionizing radiation exposure, environmental pollutants and excess alcohol consumption can also generate reactive oxygen species which may lead to cellular damages [9]. Free radicals and/or other reactive substances play an important role in the progression or the initiation of some diseases such as neurodegenrative disorders [15]. A correlation between fluoride consumption and oxidative stress in rat neural tissues has been previously reported [3].

Silymarin is a natural antioxidant agent present in fruits and seeds of *S. marianum* (milk thistle) that includes 65–80% flavonolignans with low quantity of flavonoids, fatty acids and other compounds [14]. Silybin (Fig. 1) is the major compound of

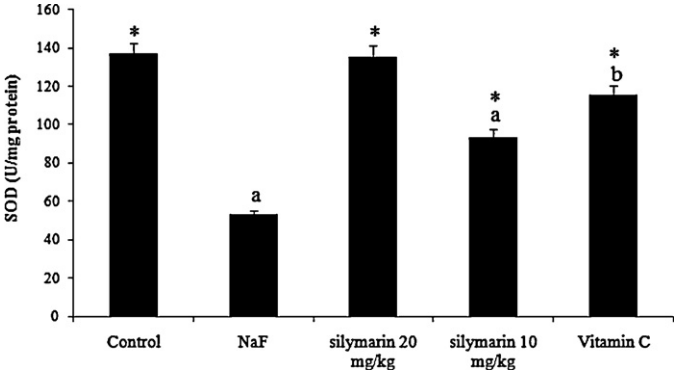

Fig. 3. Superoxide dismutase activity in the brain of experimental groups. Data are mean ± S.D. ($n = 10$). $^{a}P < 0.001$ versus control group. $^{b}P < 0.01$ versus control rats. $^{*}P < 0.001$ versus NaF.

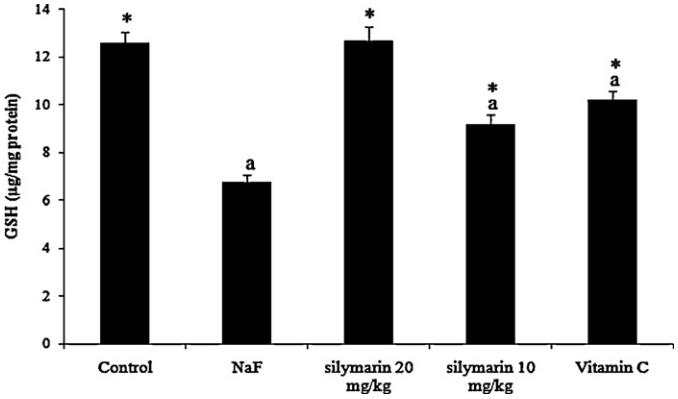

Fig. 5. Levels of reduced glutathione in the homogenates of rat brain. Data are mean ± S.D. ($n = 10$). $^{a}P < 0.001$ versus control group. $^{b}P > 0.05$ versus control rats. $^{*}P < 0.001$ versus NaF.

the silymarin mixture, and other flavonolignans of silymarin are silydianin, silychristin, isosilybin, and the flavonoid taxifolin. In the present study, the concentrations of silybin (36%) was in range of the main pharmaceutical products containing silymarin present in the US and other countries which range from 20% to 40% [16]. Analysis on chemical structures showed that flavonoids analogues with a hydroxy methyl group at position-2″ in the dioxane ring evidenced hepatoprotective activity comparable with coumarin derivatives [9]. The abundance of silybin in the silymarin composition could be the main responsible of its antioxidant effects [17]. However, it has been evidenced that a mixture of silymarin components showed higher antioxidant power than that of individual standards suggesting an important synergistic effects [18]. Numerous studies have evidenced the antioxidant effects of silybin because it inhibits radical formation in the presence of oxidative and nitrosative stress, inhibits the formation of nitric oxide, decreases the levels of lipid peroxidation, abolishes the decrease in antioxidant enzyme activities and acts as an iron chelator [19–22]. Silybin also has an anti-inflammatory action interfering with the NF-κB transduction cascade avoiding the expression of genes involved in inflammation [23,24].

According to the obtained results, sodium fluoride induced oxidative stress in rat neural tissues through significant reduction in the antioxidant enzymes activities and changes in the levels non-enzymatic antioxidants (GSH and TBARS). One week administration of silymarin prior to the fluoride intoxication is able to ameliorate the fluoride-induced toxicity in rat's brain through its antioxidant action. Several authors have previously investigated the protective potential of silymarin in diabetic [25], 6-Hydroxydopamine hemi-parkinsonian [8] and manganese [26] models of neurotoxicty. The ability of silymarin to mitigate the symptoms of these diseases may derivate from its potent antioxidant and antiradical activity as it was previously reported by several authors [9,27–29]. In particular Kiruthiga et al. [9] reported that silymarin has potent intracellular H_2O_2, $HO^{\bullet}$ and $ROO^{\bullet}$ radicals scavenging activity. In fact, silymarin can penetrate to the cells and protect them against benzo(a)pyrene-induced toxicity [9]. The present study demonstrated that silymarin treatment reduced the oxidative stress induced by sodium fluoride in rat neural tissues, supporting its antioxidant activity.

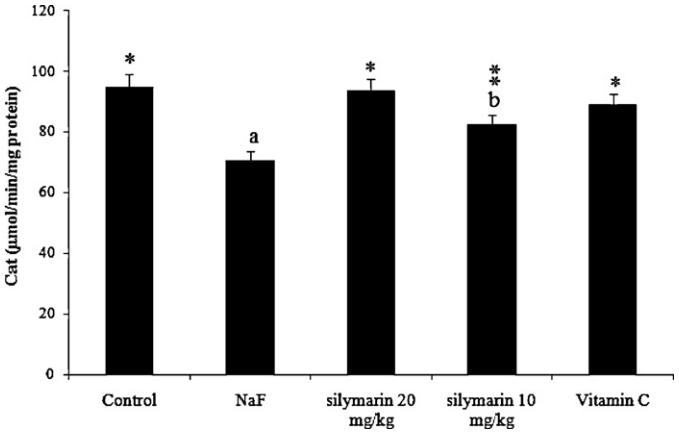

Fig. 4. Catalase activity in sodium fluoride induced oxidative stress in rat brain. Data are mean ± S.D. ($n = 10$). $^{a}P < 0.001$ versus control group. $^{b}P < 0.05$ versus control rats.* $P < 0.001$ versus NaF. **$P < 0.05$ versus NaF.

In present study, sodium fluoride exposure increased levels of TBARS and therefore the amount of lipid peroxidation in the rat's brain. This increase in lipid peroxidation levels is enhanced with iron releasing, which contributes to Fenton type reactions [30]. Sodium fluoride intoxication also caused a significant decrease in the glutathione levels, suggesting that the non-protein sulfydryl groups and protein- bound sulfydryl groups could alter each other through thiol/disulfide exchange reactions. Pretreatment with silymarin and vitamin C inhibit the sodium fluoride induced imbalance in the intracellular antioxidant defense system including both enzymatic (catalase and superoxide dismutase) and non-enzymatic (thiol based) antioxidant levels with their metabolites. Sodium fluoride intoxication decreased the superoxide dismutase activity which can result in an extra superoxide onion accumulation in rat's neural tissue. In addition, a decrease in the catalase activity may correlate with an inadequate preparation of nicotinamide adenine dinucleotide phosphate required for catalase activation [31]. Pretreatment with silymarin and vitamin C normalized the activities of the antioxidant enzymes, the levels of reduced glutathione and lipid peroxidation in neural tissues of sodium fluoride intoxicated rat.

4. Conclusion

In conclusion, this study evidenced the neuroprotective effects of silymarin against sodium fluoride-induced neurotoxicity in rat neural tissues. Administration of silymarin before sodium fluoride intoxication through drinking water reversed the antioxidant parameters altered by sodium fluoride intoxication. Further studies are needed to entirely characterize the mechanisms responsible of the silymarin neuroprotecive effects.

5. Materials and methods

5.1. Chemicals

Bovine serum albumin (BSA) and a standard protein estimation kit were purchased from ZiestChem Diagnostics 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 were purchased from Sigma–Aldrich Chemical Company (St Louis, MO, USA). The certificate of analysis purchased from Sigma–Aldrich for silymarin indicated a purity in silybin of 36%. All chemical reagents were of analytical grade or better.

5.2. Animals and experimental procedure

All experiments were performed with male Wistar rats (200–250 g) purchased from Pasteur Institute of Iran, Amol Research Center, Iran. Animals were housed in the animal room of University of Mazandaran, Babolsar, Iran. Animals were kept at room temperature and fed with pellet rat diet, and exposed to 12 h light/12 h dark cycle. Animals were allowed for two weeks to acclimatize before starting the experiments. Experiments were performed under the approval of the University of Mazandaran Institutional Animal Care and Use Committee (Approval number: No. S-2009 UMZ).

Animals were divided into the five different groups containing 10 animals each group. The first group was used as control and only received solvent intraperitoneally during 7 days. The second group was intoxicated with sodium fluoride through drinking water (600 ppm, 7 days), Groups three and four were treated with silymarin at 10 and 20 mg/kg intraperitoneally, for 7 days prior to sodium fluoride intoxication for 7 days at 600 ppm through drinking water. The animals of the group five were treated with vitamin C at 10 mg/kg intraperitoneally, for same period. Animals were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg) and sacrificed 24 h after finishing the treatment period and the brain of the animals was sampled.

5.3. Preparation of brain homogenates

The brain samples were homogenized in 10 volumes of potassium dihydrogen phosphate buffer (100 mM, pH 7.4) containing 1 mM ethylenediaminetetraacetic acid and centrifuged at 16,000 × *g*, 4 °C, for 30 min. The supernatants were recovered and immediately used for biochemical analysis.

5.4. Biochemical analysis

Lipid peroxidation levels in brain homogenates were evaluated by determination of thiobarbituric acid reactive substances (TBARS) and were expressed in terms of malondialdehyde (MDA) content, according to the method Ozturk et al. [32]. The method of Misra and Fridovich [33] was used for evaluation of superoxide dismutase (SOD) activity. One unit of SOD activity was considered as the enzyme amount required for preventing the 50% of chromogen formation in the study conditions. Catalase activity was measured by the method of Pari and Latha [34]. In this method, one unit of catalase activity was defined as the concentration of CAT which transforms 1 μmol of H_2O_2 into water under the assay conditions. Reduced glutathione levels were measured by the procedure of Ellman [35]. Different concentrations of standard solution of GSH have been used for drawing calibration curve.

5.5. Statistical analysis

The results are presented as means ± S.D. Statistical analysis was carried out using a statistical package (SPSS 17.0 for Windows®). Differences between groups were estimated using a one-way analysis of variance followed by Duncan's multiple range tests. Results were considered statistically significant when $P < 0.05$.

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