Silymarin reduced cisplatin-induced hyperalgesia by suppressing oxidative stress in male rats

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ABSTRACT

Introduction: Cisplatin is an antineoplastic agent which is used in treatment of various cancers. However its clinical use is associated with oxidative stress-mediated neuropathic pain. This research aimed to explore the effect of silymarin on cisplatin-induced hyperalgesia (CIH) and oxidative stress biomarkers in male rats.

Methods: Fifty-six male rats were allocated into seven equal groups. Hyperalgesia was caused by intraperitoneal single dose administration of cisplatin (1mg/kg) and assessed by utilizing tail-flick method. The impact of silymarin (25, 50 and 100 mg/kg/day for 15 days) on CIH was investigated on days 1, 5, 10 and 15. Blood samples were collected to assess malondialdehyde (MDA), glutathione peroxidase (GPx), superoxide dismutase (SOD) and total antioxidant status (TAS) on day fifteen.

Results: Single dose injection of cisplatin (1mg/kg) could cause a significant hyperalgesia on days 5, 10 and 15. CIH was abolished by daily administration of silymarin (50 and 100mg/kg) on days 10 and 15. Serum MDA level was decreased in cisplatin and silymarin (100 mg/kg) co-treated rats, while there was an increase in GPx, SOD as well as TAS parameters.

Conclusion: The results of this study revealed that silymarin prevents from CIH possibly by improving lipid peroxidation and oxidative stress biomarkers. Other clinical studies should be performed to establish possible use of silymarin for treatment of CIH in susceptible individuals.

Keywords:
Cisplatin
Silymarin
Hyperalgesia
Oxidative stress

Introduction

Cisplatin is an effective cytotoxic drug used for the treatment of various cancers including ovarian, testicular and bladder cancers. Its cytotoxic effect is performed by cross-linking DNA and blocking its duplication in cancer cells (Lee et al., 2015). Its clinical use is associated with neuropathic pain as a major dose limiting side effect (Khasabova et al., 2019). Several studies have proved the oxidative stress-mediated neurotoxicity of cisplatin which can lead to neuropathy and hyperalgesia. In these studies, neuronal drug transporters (Cavaletti et al., 2014), platinum DNA adducts (Dzagnidze et al., 2007; Ta et al., 2006), interference with cell cycle and mitochondrial dysfunctions (Gill and Windebank, 1998;

Lomeli et al., 2017; Podratz et al., 2011), inhibition of transcription (Todd and Lippard, 2009), altering the activity of voltage-dependent anion channel proteins (Yang et al., 2006) and oxidative stress (Areti et al., 2014; Kim et al., 2010) were found to be involved in neurotoxicity process of cisplatin. Oxidative stress-mediated neurotoxicity is one of the prominent proposed mechanisms for cisplatin-induced hyperalgesia (CIH). Studies show that cisplatin results in mitochondrial dysfunction by inhibiting mitochondrial complexes (I–IV), suppressing glutathione antioxidant activity (Kruidering et al., 1997; Siddik, 2003), augmenting the yield of reactive oxygen species (ROS) (Arany and Safirstein, 2003) and subsequent sensitization of nociceptors and spinal dorsal horn neurons (Cavaletti et al., 2014).

Silybum marianum L. that is also known as milk thistle belongs to Carduus marianum family. Historically, it has been used for treatment of various diseases such as liver and gallbladder disorders (Karimi et al., 2011; Křen and Walterová, 2005). This plant was first introduced in Europe and was found to be useful in treatment of jaundice (Mayer et al., 2005). Silymarin, active component of *Silybum marianum* L. is composed of several compounds. Among them we can point to silybin A, silybin B, isosilybin A, isosilybin B, silychristin, neosilyhermin, silyhermin and silydianin (Basiglio et al., 2009; Karimi et al., 2005b; Kaur and Agarwal, 2007). Its hepatoprotective (Morishima et al., 2010), anti-cancer (Ramasamy and Agarwal, 2008), renoprotective (Karimi et al., 2005a; Shahbazi et al., 2012), immunomodulative (Gharagozloo et al., 2010) and other therapeutic effects have been proved before (Karimi et al., 2011). Beside these effects it has been known for its positive neuronal effects. Among them we can point to its neuroprotective effects in ischemia (Hirayama et al., 2016), epilepsy (Sedaghat et al., 2017), dementia (El-Marasy et al., 2018), parkinson's disease (Baluchnejadmojarad et al., 2010b; Haddadi et al., 2015; Haddadi et al., 2013; Haddadi et al., 2018; Haddadi et al., 2014) depression (Karimi and Saradeghi Keisari, 2007) and prevention of diabetic neuropathy (Baluchnejadmojarad et al., 2010a).

Literature review reveals that there is a little published data over the neuroprotective effect of silymarin on CIH. Thereby, this study was conducted to investigate the attenuating effects of silymarin on CIH and to assess involvement of oxidative stress biomarkers in the observed effects.

Materials and methods

Chemicals

All chemicals were purchased from Sigma Chemical Co. (Germany). Silymarin was donated by Goldaru Pharmaceutical Company (Esfahan, Iran). Drugs solutions were prepared freshly on the days of experiment by dissolving in physiological saline (0.9% NaCl) except for silymarin which was dissolved in 50% polyethylene glycol 400 (PEG). The drugs were given intraperitoneally (IP) on days of experimentation.

Animals

The study was carried on 56 male Wistar rats weighing 180–200g. The rats were allocated randomly in seven equal groups and housed in standard Plexiglass cages (4 rats per cage) at room temperature (22±3°C) and a 12-h light period. The study was carried out in conformity with the ethical guidelines of regional research ethic committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.1056).

Induction of hyperalgesia

In order to induce hyperalgesia, single dose of cisplatin (1mg/kg, IP) was injected to the rats and then pain latency time was assessed on days 1 (30min after cisplatin injection), 5, 10 and 15 by utilizing the tail-flick test until significant hyperalgesia was detected.

Tail-flick test

The tail-flick method was utilized for quantifying pain feeling 30 minutes after drugs administration. Briefly, heat-producing beam was operated to the distal 3cm of the tail by using a tail-flick apparatus (Mod.37360, UGO BASILE, Italy) with an irradiation intensity of 20mW/cm². In order to avoid further tissue damage twenty seconds was considered as a test cutoff time. The latency time for withdrawal of the tail from the painful heat-producing beam was recorded on days 1, 5, 10 and 15.

Sample preparation

At the end of behavioral pain study (day 15), blood samples (n=8 per group) was collected rapidly from the carotid arteries and then centrifuged at 3500rpm for 10min. The serum and heparinized whole blood samples

were collected and stored at −80°C until biochemical estimation.

Malondialdehyde (MDA) assay

The amount of MDA as a major end product of oxidative damage was measured by double heating method. Briefly, serum samples were mixed with trichloroacetic acid to precipitate protein, followed by centrifuging and collecting the supernatant. The supernatant reacted with an equal volume of thiobarbitoric acid in 90−100°C for 10min. The absorbance was measured spectrophotometrically at 532nm and MDA level was reported as nMol/ml.

Glutathione peroxidase (GPx) assays

The GPx activity in heparinized whole blood samples was scaled according to instructions of commercially available GPx kit (Ransel, Randox laboratory, UK). The GPx activity was quantified at 340nm and expressed as Unit/g hemoglobin. The method was first introduced by Paglia and Valentine (1967). During the procedure, GPx catalyzes the oxidation of glutathione by cumene hydroperoxide. Glutathione Reductase and NADPH help the oxidized glutathione to be rapidly transformed into the reduced form. By measuring the absorbance, the amounts of the products (resulting from the reactions) and consequently GPx would be estimated.

Superoxide dismutase (SOD) assay

Activity of SOD was estimated in heparinized whole blood samples as described by commercially available SOD kit (Ransod, Randox laboratory, UK). Intensity of the color was quantified spectrophometrically at 505nm. The data of SOD estimation were shown as Unit/g hemoglobin. In this method, xanthine and xanthine oxidase are used for generating superoxide radicals. Reaction between the radicals and 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride results in the formation of red formazan dye. The higher the activity of SOD, the less red formazan is formed (Woolliams et al., 1983; Suttle, 1986; Suttle and McMurray, 1983; Arthur and Boyne, 1985).

Total antioxidant status (TAS) assay

The TAS measurement was performed according to instructions of TAS kit (Randox laboratory, UK).

Absorbance was measured by spectrophotometer at 600nm and TAS results were expressed as mMol/l. In this method, metmyoglobin and H2O2 turn ABTS® (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) to its radical cation form which is blue-green in color. Endogenous and exogenous antioxidants would attenuate this color formation depending on their concentrations and the extent of this attenuation would be a criterion for evaluating their antioxidant capacity (Miller et al., 1993).

Data analysis

Statistical analysis was performed using SPSS software (version 16). The results were shown as mean±SEM values and compared by one-way analysis of variance. Significant difference was considered at the level of $P<0.05$. Tukey multiple comparison test was used to find means that are significantly different from each other.

Results

Effect of cisplatin on pain threshold

In three groups of normal, saline (1ml/kg, IP) and cisplatin-treated (1mg/kg, IP) animals, response to the noxious stimulus was assessed on days 1 (30min after drugs administration), 5, 10 and 15 by using tail flick apparatus. Results showed that cisplatin induced significant ($P<0.05$) pain on days 5, 10 and 15 (Figure 1).

Effect of silymarin on CIH

Tail flick latency time was assessed in normal, vehicle (50% PEG 400, 1ml/kg, IP), cisplatin (1 mg/kg, IP) and three cisplatin+sliymarin (25, 50 and 100mg/kg, IP) co-administered groups. As it has been shown in Figure 2, in those groups of rats that were co-treated with cisplatin and silymarin there was a significant ($P<0.05$ and 0.01) increase in pain latency time on days 10 and 15, when compared with cisplatin received rats. The greatest effect was observed with 100mg/kg silymarin ($P<0.01$).

Assessment of oxidative stress parameters

At the end of pain study, the MDA, GPx, SOD and TAS as oxidative stress parameters were assessed in normal, cisplatin and cisplatin+silymarin (100mg/kg) co-treated rats (Figure 3). In cisplatin treated rats the amount of MDA was increased ($P<0.05$) in comparison to normal animals; while we observed a significant decrease
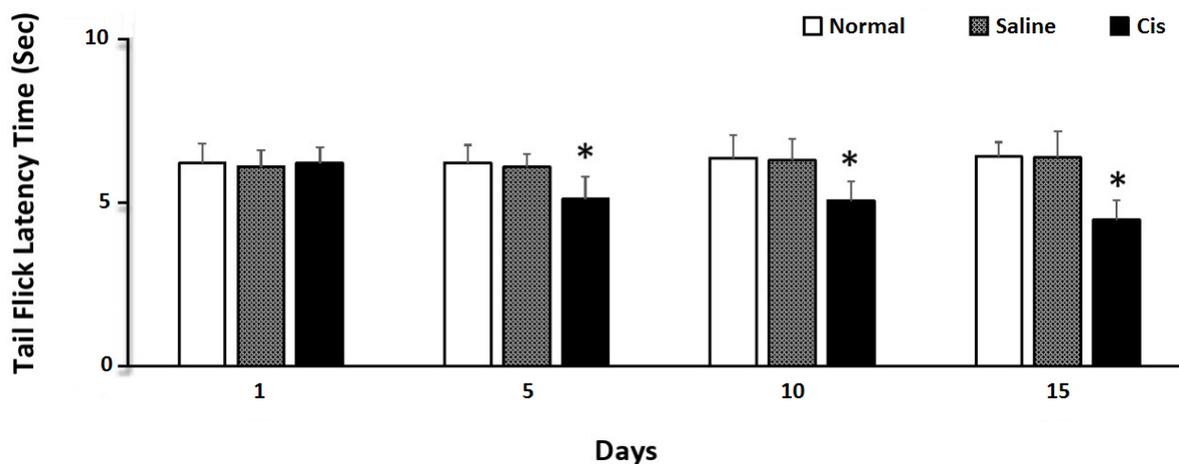
FIGURE 1. Effect of cisplatin (Cis, 1mg/kg, IP) on tail-flick latency time (s). Data has been shown as mean±SEM, n=8 rats per group. *P<0.05, when compared with normal group.
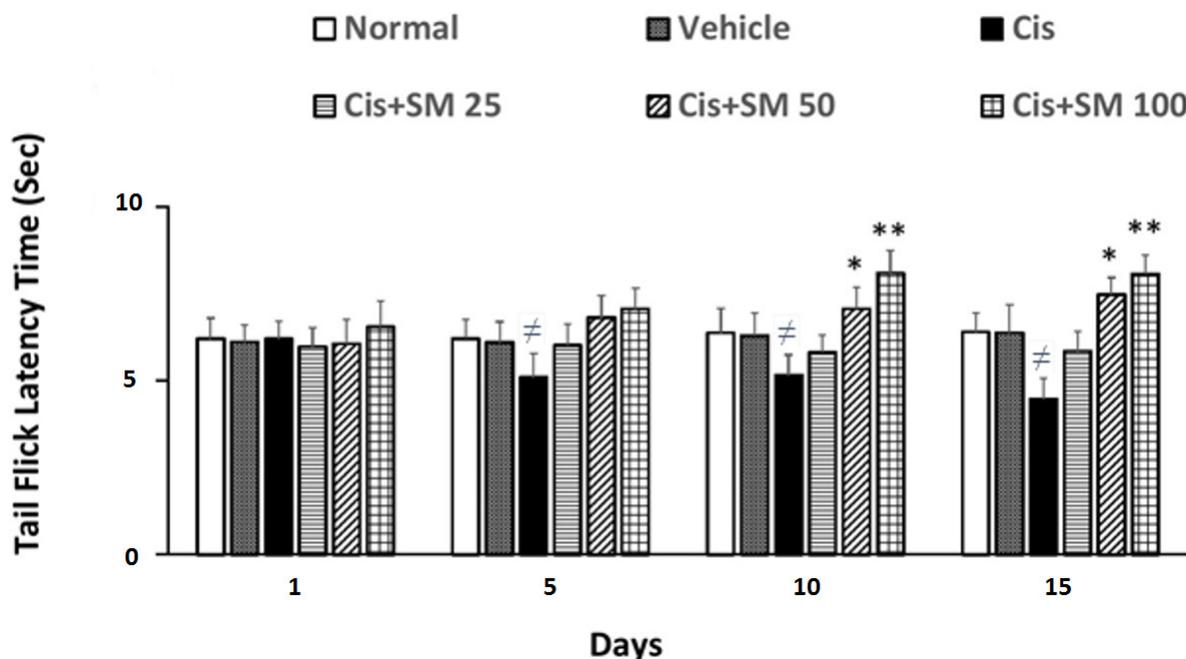

FIGURE 2. Effect of silymarin (SM, 25, 50 and 100mg/kg, IP) on cisplatin (Cis, 1mg/kg, IP)-induced hyperalgesia. Data has been shown as mean±SEM, n=8 rats per group. ≠P<0.05 when compared with normal group; *P<0.05 and **P<0.01, when compared with Cis group.

(P<0.01) in MDA level in cisplatin and silymarin co-administered animals. Furthermore, as shown in Figure 3, cisplatin significantly reduced GPx, SOD and TAS compared to control group (P<0.05); while silymarin was able to reverse all these alterations (P<0.01).

Discussion

In cancer therapy, most of the cytotoxic drugs are capable to induce peripheral nervous system toxicity and painful neuropathy as an unwanted affect (Paice, 2003). Cisplatin is one of the commonly used anticancer drugs that causes peripheral neuropathic pain in a dose-

and time-dependent manner (Joseph and Levine, 2009). The pain develops during 4 months and spontaneous attenuation occurs after a while, nevertheless it does not disappear completely after cessation of treatment (Authier et al., 2000). Results of an animal study revealed that mechanical allodynia and hyperalgesia last for 15 days after cisplatin injection (Pacharinsak and Beitz, 2008). In our study, cisplatin (1mg/kg) caused remarkable hypersensitivity to thermal stimulus on days 5, 10 and 15. Afterward, this dose of cisplatin was used for producing hyperalgesia throughout the study. Cisplatin was administered in a single dose and
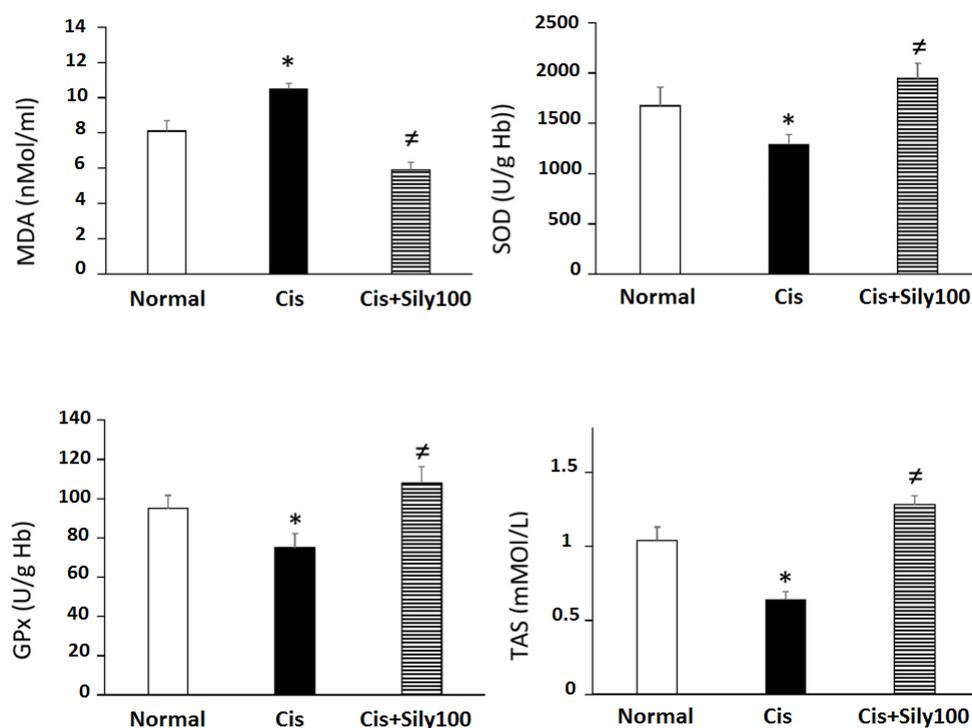
FIGURE 3. The levels of malondialdehyde (MDA, upper left), superoxide dismutase (SOD, upper right), glutathione peroxidase (GPx, lower left) and total antioxidant status (TAS, lower right) in normal, cisplatin (Cis, 1mg/kg, IP), cisplatin and silymarin (SM, 100mg/kg/day, IP) co-treated rats. Data has been shown as mean±SEM, n=8 rats per group. [*]$P<0.05$, when compared with normal group; [≠]$P<0.01$, when compared with Cis group.

decreased the pain threshold in the animals over time so that they would react to tail-flick test faster than normal animals. The mean latency time was lowered day by day, so the most prominent decrease was observed on day fifteen. This observation is in accordance with previous studies showing CIH (Balayssac et al., 2009; Nayebi et al., 2012).

Oxidative stress reactions are among underlying mechanisms of CIH. Free radicals such as superoxide, hydroxyl, hydrogen peroxide and other radicals produced by lipid peroxidation can exacerbate oxidative stress reactions, nerve tissue damages and as a result, could cause hyperalgesia (Kittur et al., 2002; Namvaran-Abbas-Abad and Tavakkoli, 2012). Therefore, the use of naturally occurring antioxidant compounds for attenuating cisplatin-induced neuropathic pain would be justifiable.

In recent years, lots of studies have been focused on the concept of pain (Luo et al., 2020; Parvizpur et al., 2020; Majidi et al., 2018). In this study, a natural compound which is extracted from an herb was evaluated. Silymarin is a natural extract of *Silybum marianum* containing several flavonolignans such as silybin A, silybin B, isosilybin A, isosilybin B, silychristin and silydianin

(Surai, 2015). For its antioxidant activity, we can refer to increase of cellular glutathione, superoxide dismutase, glutathione peroxidase and catalase in rat brain (Manna et al., 1999). In our study, silymarin decreased CIH dose dependently so the most prominent effect was obtained at dose of 100mg on day fifteen. Previous *in vivo* studies have proved the antinociceptive effects of silymarin and suggested its anti-inflammatory and antioxidant properties as the underlying mechanisms. Silymarin-induced inhibition of ROS and inflammatory mediators were noted in some studies (Hassani et al., 2015; Jadhav and Upasani, 2009; Sahib, 2011). In our study estimation of oxidative stress parameters indicates a significant increase and decrease in antioxidant (SOD, GPx and TAS levels) and oxidative (MDA) parameters activity respectively in cisplatin and silymarin co-treated group on day fifteen. It seems that effect of silymarin on improving of CIH may be related to its potential antioxidant activity. It has been shown that the analgesic effects of antioxidant compounds like silymarin in the pain thermal test can be due to their inhibitory effect on the 2-lipoxygenase enzyme (Hunt et al., 2001; Vargas-Mendoza et al., 2014). Thus suppression of inflammatory mediators subsequent to silymarin antioxidant activity is

not negligible.

Conclusion

According to our results, we suggested that silymarin may be useful in attenuating of CIH possibly through improving stress oxidative biomarkers. However, further investigations should be performed to explain its exact mechanism of action and efficacy in treating CIH.

Acknowledgments

Conflict of interest

The authors declared no conflict of interest for this study.

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