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EFFECT OF *P*-COUMARIC ACID AGAINST OXIDATIVE STRESS INDUCED BY CISPLATIN IN BRAIN TISSUE OF RATS

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ABSTRACT

The antioxidant effect of *p*-Coumaric acid was evaluated against brain damage induced by cisplatin in rats. In this experimental study, twenty-four Sprague-Dawley rats were divided into four groups; control, *p*-Coumaric acid, cisplatin, and *p*-coumaric acid+cisplatin. Cisplatin was administered intraperitoneally (i.p) in a single dose of 10 mg kg^{-1}. *p*-Coumaric acid was given in a doses of 100 mg kg^{-1}i.p. for three days. In the cisplatin group, rats were sacrificed under anesthesia after 72 hours administration of cisplatin and brain tissues were removed. To assess the changes in biochemical aspects, activities of superoxide dismutase and level of malondialdehyde, glutathione were measured. An increase on activities of superoxide dismutase and level of glutathione has been observed in the group applied *p*-coumaric acid+cisplatin when compared with the cisplatin group. Moreover, the *p*-coumaric acid+cisplatin group had lower level of malondialdehyde compared to that of the cisplatin group. In conclusion, our results indicated that *p*-coumaric acid can mitigate cisplatin-induced oxidative damage in brain tissue.

Keywords: *p*-Coumaric acid, Cisplatin, Oxidative Stress, Brain, Rats

INTRODUCTION

Cisplatin has been known as an antineoplastic drug used effectively in the treatment of numerous tumors, including head neck, esophageal, testicular, ovarian and bladder cancer. Neurotoxic effects induced by cisplatin includes increased oxidative-nitrosative stress, proinflammatory cytokines, mitochondrial dysfunction, DNA damage and apoptotic cell death resulting in multiple morphological changes in the neurons such as axonal shrinkage and demyelination (Tuncer *et al*., 2010). Besides these, it also targets the neural progenitor cells in the hippocampal area andsuppresses neurogenesis and cell proliferation (Piccolini *et al.*, 2012).

Phenolic compounds as secondary plant metabolites are mostly found in plant food products. These compounds are considered to be indispensable parts of both human and animal diets (Bursal *et al.,* 2013; Gülçin and Beydemir, 2013; Topal *et al.*, 2017). It has been reported that the most known groups of natural antioxidants contain tocopherols, flavonoids and phenolic acids (Arabaci *et al.*, 2014; Topal *et al.*, 2014; Çakmakçı *et al.*, 2015).

p-Coumaric acid is known as a compound in the polyphenolic structure found in many plants and vegetables in nature (Innocenti *et al.*, 2010; Öztürk Sarıkaya *et al.*, 2010). It mostly exists in fruits e.g. apple and pears and within various plants such as beans, potatoes, tomatoes and tea (Gülçin *et al.*, 2010a). In addition to these, *p*-coumaric acid is also found in pineapple. There is data showing that *p*-coumaric acid decreases developmental risk of some cancer types such as stomach cancer (Şerbetci *et al.*, 2010).

Several antioxidant molecules such as coenzyme Q10 (Machado *et al.*, 2013) cyaniding (Li *et al.*, 2015), walnut (Shabani *et al.*, 2012), curcumin (Mendonca *et al.*, 2013), have been defined to prevent cisplatin and its derivatives-induced neurotoxicity in the experimental models. Based on significant protective profiles of *p*-Coumaric acid, it is worthwhile to evaluate its neuroprotective potential against cisplatin-induced neurotoxicity. Therefore, this study was planned to evaluate the attenuating effect of *p*-coumaric acid on cisplatin induced-neurotoxicity by measuring the oxidative stress marker malondialdehyde (MDA) and level of antioxidants such as superoxide dismutase (SOD) and glutathione (GSH).

MATERIALS AND METHODS

Chemicals: *p*-Coumaric acid was purchased from Sigma Chemical Co., St. Louis, MO, USA. Cisplatin, in the form of Cisplatin-Koçak 50 was obtained from Koçak Farma Co., İstanbul, Turkey. Pentothal sodium (0.5g Thiopental

sodium) was purchased Ulagay İlaç Sanayi, İstanbul, Turkey.

Animals and Diet: The study was conducted on healthy Sprague-Dawley type rats weighing 230±10 g, purchased from the Central Research of Experimental Animals, Atatürk University, Turkey. The rats were kept in cages, renovated every 24 h under a 12/12 h light: dark cycle, 60±5% humidity with average temperature of 22 °C. The rats were fed a standard pellet diet throughout the experiment. The rats had free access to water and feed. The experiment was conducted according to the guidelines approved by Experimental Animals Local Ethical Committee of Atatürk University (Approval No. 95; Dated. 26th May, 2016).

Experimental Design: After weighing, the rats were equally divided into 4 groups; each consists of 6 rats per group. The rats in the control group were fed basal diet without any drug. The animals in the p-coumaric acid group were applied treatment with p-coumaric acid (100 mg.kg^{-1} body weight) daily for a period of 3 days. p-Coumaric acid was dissolved in 20% ethanol solution. Cisplatin group contained rats that were injected cisplatin intraperitoneally at the single dose of (10 mg.kg^{-1} body weight) on the first day of experiment. In the p-coumaric acid+cisplatin group, *p*-Coumaric acid intraperitoneally at the dose of 100 mg.kg^{-1} body weight were administered and then subsequently injected with cisplatin (10 mg.kg^{-1} body weight; intraperitoneally). After 72 h of cisplatin injection, the rats were sacrificed using thiopental sodium (25 mg.kg^{-1} body weight). Brain tissues were quickly cut and washed in ice-chilled normal saline and were kept at -80 °C until analyzed.

Biochemical Analysis: Superoxide dismutase (SOD) activity glutathione (GSH) and malondialdehyde (MDA) levels were measured at room temperature using ELISA reader according to methods by Sun (1988), Sedlak (1968) and Ohkawa (1979) respectively; Data of the SOD activity, GSH and MDA levels in the tissues are presented as U/mg protein and nmol/mg protein respectively. Protein concentrations were defined by the Lowry method (1951) using commercial protein standards (Total protein kit-TP0300-1 KT; Sigma Chemical Co., Munich, Germany).

Statistical Analysis: Data expressed as mean ± SD. All results were analyzed employing one-way analysis of variance. Differences among the groups were analyzed by the Duncan's multiple range tests. The significance level was determined at $p < 0.05$.

RESULTS AND DISCUSSION

The brain is a very complex and sensitive organ, which is greatly affected by several chemotherapeutic agents used to treat cancers. Several studies reported that neuro-toxicants negatively influence the hippocampus by activating the neuro-inflammatory cascade, which in turn resulted in behavioral deficits (Jangra *et al.*, 2015). Although chemotherapy is one of the most effective strategies for the treatment of cancer, however the used drugs often causes several adverse effects such as cardiotoxicity, ototoxicity, nephrotoxicity and neurotoxicity, thereby limiting its clinical application (Li *et al.*, 2006; Kwatra *et al.*, 2016). Cisplatin drug is hydrophilic in nature, therefore cannot transit through the blood-brain barrier under normal physiological conditions. However, under altered physiological milieu such as hypoxia, cisplatin is neurotoxic owing to its capacity to cross the blood brain barrier by altering its structure (Oz *et al.*, 2015; Abou-Elghait *et al.*, 2010). Cisplatin eventually damages cerebral, cerebellar cortex, and hippocampus, which ultimately results in seizures, cognition deficits, peripheral and autonomic neuropathies (Chtourou *et al.*, 2015; Harandi *et al.*, 2015).

So far, several mechanisms of the cisplatin-induced neurotoxicity have been proposed. Some of these include generation of reactive oxygen species (ROS) and consumption of enzymatic and non-enzymatic antioxidants in brain tissue. Reactive oxygen metabolites contain superoxide anion, hydrogen peroxide and hydroxyl radicals, which are significant intermediary of tissue or organ injury (Gülçin *et al.*, 2010b; 2010c). These radicals result in peroxidation of polyunsaturated fatty acids in membranes (Reddy *et al.*, 2016) with a concomitant rise of MDA and reduced glutathione levels (GSH). Specifically in brain tissue, ROS affect the electron transfer chain in mitochondria and damage the nuclear and mitochondrial DNA resulting in cell death (Marullo *et al.*, 2013). Numerous studies have been carried out indicating the antioxidant activities of phenolic compounds (Gülçin *et al.*, 2006a; 2006b; 2007; Ak and Gülcin, 2008). These include linking of metal ions, scavenging of ROS, arrangement of endogenous antioxidant enzymes, or protection against oxidative damage of biological structures (Ursini *et al.*, 1999; Gülçin *et al.*, 2009a; Ekinci-Akdemir *et al.*, 2016a; Ekinci-Akdemir *et al.*, 2016b). Many researches have demonstrated the relationship between phenolic acids intensive diets and the protection from various diseases (Morton *et al.*, 2000; Gülçin *et al.*, 2009b). *p*-Coumaric acid as a phenolic acid has been reported to reduce the risk of stomach cancer (Scalbert *et al.*, 2000).

Results of the present study revealed that the cisplatin-treated group was presented with reduced SOD and glutathione levels in the brain tissue with a concomitant increase of MDA levels when compared with the control group (Table 1). Treatments with *p*-coumaric acid+cisplatin alleviated these cisplatin-induced changes and reduced the level of MDA in brain tissue, which was comparable to the control group (Table 1).

Investigations of the current study further strengthen the already documented mechanisms of neurotoxicity. During the current situation reduction in SOD activity after cisplatin application might be due to loss of zinc and copper, which are imperative for the enzyme efficiency (Sharma *et al.*, 1985). Reduced SOD activity results in inadequacy to scavenge the superoxide anion produced during the normal metabolic process. Furthermore, reduction in GSH level of cisplatin treated group also resulted in diminished capability of the brain tissue to scavenge hydrogen peroxide and lipid peroxide products. Results of our study showed that *p*-Coumaric acid attenuated the demolition of GSH levels and antioxidant enzyme activity in the brain tissue of animals administered with cisplatin and ensured preservation the brain tissue. We can say that *p*-Coumaric acid maintained against oxidative damage of the brain tissue induced by cisplatin.

Table 1. The effects of *p*-coumaric acid treatment on superoxide dismutase and glutathione levels in cisplatin-induced damage in brain tissue.

Groups	Control	Cisplatin	*p*-Coumaric Acid	Cisplatin+*p*-Coumaric Acid
SOD (U/mg protein)	14.00±3.39^{c}	5.53±0.76 ^{a}	12.60±2.20^{b,c}	10.42±1.83 ^{b}
GSH (nmol/mg protein)	3.83±0.76 ^{c}	1.50±0.37 ^{a}	3.31±0.60^{b,c}	2.69±0.48 ^{b}

$^{a-c}$Means within a row with different superscripts differ significantly ($p < 0.05$)

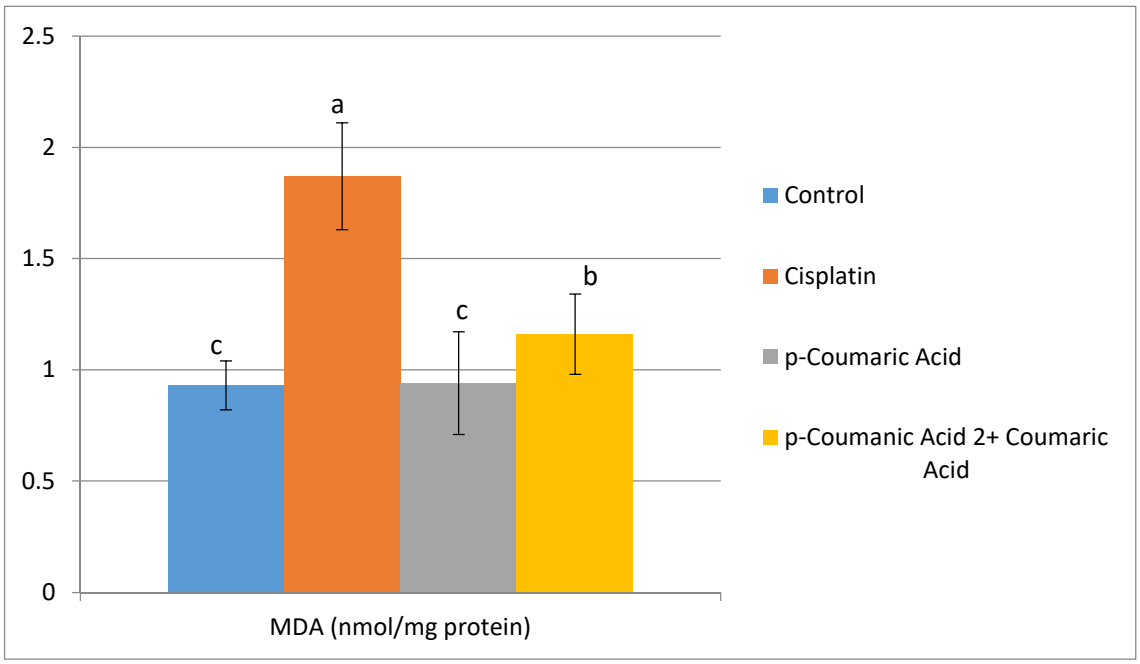

Figure 1. The effects of *p*-coumaric acid treatment on malondialdehyde (MDA) level in cisplatin-induced damage in brain tissue; $^{a-c}$Different superscripts in line differ significantly ($P < 0.05$)

In conclusion, the neurotoxicity of cisplatin was attenuated by *p*-coumaric acid, a derivative of phenolic acid in experimental model.

Conflict of interest: The authors declare no conflict of interest. This study was presented as an abstract presentation in International Science Symposium 1-4 September 2016, İstanbul, Turkey.

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