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# Renal protective effects of arjunolic acid in a cisplatin-induced nephrotoxicity model

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ABSTRACT

Cisplatin is the first platinum-containing anti-cancer drugs. Cisplatin notable side effect of nephrotoxicity limits its use in clinic. Meanwhile, arjunolic acid possesses anti-inflammatory properties and plays protective roles against chemically induced organ pathophysiology. This study was conducted to find out whether arjunolic acid could attenuate kidney damage in rats, and to elucidate its possible mechanism of action. Fifty rats were treated with cisplatin (10 mg/kg) in the presence/absence of 100 or 250 mg/kg arjunolic acid. Arjunolic acid is given 1 h after cisplatin. Morphological changes were assessed in kidney sections stained with Hematoxylin/Eosin and Masson Trichrome. Kidney samples were used for measurements of transforming growth factor (TGF)-β1 and its type 1 receptor (TGF-βR1), tumor necrosis factor (TNF)-α and interleukin (IL)-1β by ELISA. Gene expression NFκB was determined by real time-PCR. Kidney tissue apoptosis was assessed by measuring the activities of caspase-3/8/9. The renal protective effect of arjunolic acid was confirmed by approximately normal appearance of renal tissue and the relatively unaffected serum creatinine and urea levels. Furthermore, arjunolic acid showed dose dependent reduction in cisplatin-induced elevation in renal levels of TGF-βR1, TGF-β1, TNF-α, IL-1β and caspases. These findings demonstrated that arjunolic acid attenuates cisplatin nephrotoxicity either indirectly by enhancing body antioxidant activity or directly through several mechanisms, including inhibition of pro-inflammatory cytokines, blocking activation of TGF-β1, and anti-apoptotic effects.



## 1. Introduction

Cisplatin (cis-diamminedichloroplatinum II) is a valuable and potent platinum-based medication used either alone or in combination with other agents in most chemotherapy regimens for solid or hematologic tumors [29]. Cisplatin induces the formation of DNA intra-strand crosslinks. Cisplatin–DNA cross links cause cytotoxic lesions in both tumours and other dividing cells [1]. Hence, the clinical application of cisplatin is limited by tumor cell resistance and myriad of adverse effects such as nephrotoxicity, ototoxicity, neurotoxicity and emetogenicity [24].

The major side effect of cisplatin is dose-dependent nephrotoxicity, which limits the amount of drug that can be administered. Nephrotoxicity occurs in 25–42% of patients treated with cisplatin [29]. Cisplatin accumulates largely in the kidneys more than any other tissues and this explains the high susceptibility of the kidneys to cisplatin. Its renal toxic effects occur primarily in the proximal tubule, particularly in S3 segment of the tubular epithelial cells; followed by glomeruli and distal tubules [36]. Renal function deterioration manifests as decline in glomerular filtration rates (GFR), diminished renal blood flow, increased levels of creatinine, hypomagnesemia, and hypokalemia [12]. Despite extensive studies, the mechanism underlying cisplatin-induced nephrotoxicity is not fully understood, but is hypothesized to be complex and multi-factorial [43]. These proposed mechanisms include the accumulation of cisplatin in kidney cells with direct tubular toxicity, its subsequent conversion to nephrotoxins, generation of reactive oxygen species (ROS), inflammation, DNA damage, mitochondrial dysfunction and activation of apoptotic pathways [26].

A number of strategies have been proposed to prevent or attenuate cisplatin-induced nephrotoxicity. However, none of these strategies proved to show full effectiveness [19]. Although hydration protocols showed beneficial effects with reduction of cisplatin-induced nephrotoxicity, still about one-third of the

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patients receiving cisplatin combined with hydration show signs of renal damage [45]. Hence, the need for safe and effective alternatives that target various molecular mechanisms of cisplatin-induced nephrotoxicity is imperative.

Arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid) is a naturally occurring chiral pentacyclic triterpenoid saponin and a main constituent of the bark of *Terminalia arjuna* [22]. It has been shown that arjunolic acid possesses multi-functional biological activities, including anti-fungal [30], anti-bacterial [11], anti-inflammatory [32] and hepatoprotective [35]. Interestingly, it protects many body organs against toxicity induced by various drugs and chemicals [20,33]. In this context, we recently demonstrated cardioprotective effect of arjunolic acid against sodium nitrite-induced cardiac damage in rats [3].

Despite large number of studies, the exact mechanisms underlying its beneficial therapeutic effects still lacks substantial data. Arjunolic acid was previously reported to attenuate cisplatin-induced nephrotoxicity primarily by acting on tubular epithelial cells in kidney preventing its degeneration and atrophy. Interestingly, a recent study has addressed arjunolic acid role in cisplatin-induced nephrotoxicity through inhibition of kidney injury marker, Kim-1 and B cell lymphoma-2 (Bcl-2) [41]. On the basis of these data, the present study was designed to investigate possible inhibitory effects of arjunolic acid on cisplatin-induced elevation of renal inflammatory, fibrosis and apoptosis markers.

## 2. Materials and methods

### 2.1. Study medication

Arjunolic acid, naturally occurring chiral pentacyclic triterpenoid saponin and a main constituent of the bark of *T. arjuna*, was purchased from Hangzhou Dayangchem (Zhejiang, China, purity 98%, LD 50 = 980 mg/kg).

### 2.2. Animal preparation and experimental design

The animal protocol was approved by the ethical committee of the Faculty of Pharmacy, University of Mansoura. The adopted guidelines were in accordance with the Principles of Laboratory Animals Care [8]. Male, Sprague Dawley rats weighing 100–150 g were used. All animals in the study were maintained under standard conditions of temperature, about 25 °C, with a regular 12-h light/12-h dark cycle, and allowed free access to food and water. Rats were fed with standard rat food. Nephrotoxicity was induced by 10 mg/kg cisplatin, ip, single dose (Merck Co, Whitehouse Station, NJ, USA) [5]. This evoked a significant increase in serum creatinine levels after seven days from the start of the experiment (three days after the cisplatin injection). Rats were classified into the following groups, with 10 rats in each:

*Group (1):* received 0.2 ml of intraperitoneal (ip) of phosphate-buffered saline (PBS, 10 mM, pH 7.4), on the 1st, 4th and the 7th day of treatment, and served as the untreated control group.
*Group (2):* received 100 mg/kg arjunolic acid by oral gavage. The animals received arjunolic acid, on the 1st, 4th and the 7th day of treatment, and served as the treated control group.
*Group (3):* received cisplatin (10 mg/kg, ip, on the 4th day of the experiment), 1 h prior to the oral gavage of 0.2 mL of PBS on the 1st, 4th and the 7th day of treatment.
*Group (4):* received cisplatin (10 mg/kg, ip, on the 4th day of the experiment), 1 h prior to oral gavage of arjunolic acid (100 mg/kg). The animals received arjunolic acid, on the 1st, 4th and 7th day of treatment.
*Group (5):* received cisplatin (10 mg/kg, ip, on the 4th day of the experiment), 1 h prior to oral gavage of arjunolic acid (250 mg/kg). The animals received arjunolic acid on the 1st, 4th and 7th day of treatment.

The doses and time course of experiments in this study were in the range of those used in other studies involving the same animal species [6,13,14]. The doses and the route of drug administration of arjunolic acid were in accordance with those used in other studies applied for the same animal species [3]. In addition, the dose was confirmed after appropriate preliminary experiments.

### 2.3. Collection of samples

At the end of the specified experimental period (7 or 10 days), animals were anesthetized, blood samples were collected and serum samples were separated. Finally, the animals were sacrificed and kidneys were removed. The right kidney was fixed in 10% buffered formalin for subsequent morphological analysis; the left one was used to make a 10% (w/v) homogenate in phosphate-buffered saline (PBS, pH 7.4) [9]. The homogenate was centrifuged at 3000 revolution/minute for 10 min at 4 °C and the supernatant was removed and stored at −80 °C for further assay.

### 2.4. Morphological analysis of renal tissue

The right kidney was cut longitudinally; one-half was fixed in 10% buffered formalin and embedded in paraffin. Five micrometer-thickness sections were cut. At least two different sections were examined per kidney sample in random order and masked mannar. A total of four sets of slides were prepared for the following histopathological evaluation:

*Mayer's hematoxylin and eosin (H/E)* for examination of cell structure with a light microscope [6].
*Masson's Trichrome stain* was used for the assessment and calculation of area and percentage of fibrosis. Collagen and the other extracellular matrix components (ECM) were also evaluated in Masson's Trichome stained slides. Areas of fibrosis appears as blue and parenchyma as red. For calculation of area and percentage area of fibrosis, the slides were photographed using Olympus digital camera installed on Olympus® microscope with 0.5× photo adaptor, using objective lens ×40. The images were then analyzed on Intel® Core® 13 based computer using Video Test Morphology®software (Russia) with a specific built-in routine for stain quantification.

### 2.5. Measuring renal glomerular function

Serum urea and creatinine were measured kinetically in rat serum. Kits from Dp International Co. were used

### 2.6. Measuring oxidative stress in renal homogenate

Oxidative stress was evaluated by measuring the following parameters in renal homogenates:

*NADPH oxidase activity* was determined using cytochrome C reductase (NADPH) assay kit (Sigma Aldrich Chemicals Co., St. Louis, MO, USA) in accordance with the manufacturer protocol. One unit of enzyme activity will reduce 1 μmol oxidized cytochrome C in the presence of 100 μmol NADPH per minute at pH 7.8 at 25 °C.
*Renal lipid peroxides measured as MDA* were determined in accordance with the reported method of Satoh [40], using a kit from Biodiagnostic Company.