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# Renoprotective effect of the isoflavonoid biochanin A against cisplatin induced acute kidney injury in mice: Effect on inflammatory burden and p53 apoptosis

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ABSTRACT

Cisplatin is a potent widely-used chemotherapeutics; however, its clinical use is associated with nephrotoxicity. Renoprotective approaches are being discovered to halt the tubular cell death due to inflammatory and apoptotic burdens. In the present study, the renoprotective effects of different doses of biochanin A (10, 20 or 40 mg/kg) in mice treated with a single injection of cisplatin (10 mg/kg) were reported. Cisplatin administration resulted in marked increases in serum creatinine and blood urea nitrogen. Further, renal homogenates showed increased level of inflammatory cytokines and upregulation of the expression of p53 up-regulated modulator of apoptosis (PUMA), p53 and caspase 3 but downregulation in Nrf2 expression. Furthermore, cisplatin group showed marked necrosis and degenerated tubular lining epithelial cells with frequently detected apoptotic bodies. Mice treated with biochanin A (10, 20 or 40 mg/kg) for 14 days prior to cisplatin abrogated cisplatin-mediated damage. Furthermore, the elevated serum creatinine and urea levels were lessened by some doses of biochanin A, indicating protection against renal injury. Similarly, the changes in apoptosis and inflammatory markers have ameliorated to significant levels ($P < 0.05$). The results suggest biochanin A as a nephroprotective agent against cisplatin toxicity. Overall, this nephroprotective effect of biochanin A involved anti-inflammatory and anti-apoptotic activities.

## 1. Introduction

An inorganic platinum compound, cisplatin (cis-diaminedichloroplatinum II) is one of the most effective and active cancer chemotherapy applied in the management of various malignancies of both pediatric and adult [1]. Various types of solid tumors, including cancers of the bladder, lung, endometrium, head and neck, testis, ovary and cervix have been extensively treated by cisplatin [2]. The anticancer activity of cisplatin is believed to result from its interactions with DNA. The drug binds with N7 of purine bases forming monoadducts [3]. Cisplatin–DNA adducts can inhibit fundamental cellular processes, such as replication [4] and DNA repair [5].

Despite its importance for cancer therapy and possessing of various chemotherapeutic properties the clinical use of this drug is actually diminished by toxic acute kidney injury or progressive and a dose-dependent nephrotoxicity, which occasionally demanding discontinuation of treatment or a dose reduction and otherwise it may threaten life [6,7]. Clinically, cisplatin nephrotoxicity manifested as low glomerular filtration rate, increased serum creatinine level and depletion of serum levels of potassium and magnesium and is often seen after 10 days of cisplatin administration [8–10]. A common histopathological feature of cisplatin nephrotoxicity is the renal tissue damage which characterized

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by tubular cell death in the form of apoptosis and necrosis [7].

The mediators and molecular mechanisms associated with inflammatory injury in either ischemic or toxic acute renal failure still under investigation. Cisplatin-induced injuries contain oxidant stress [11,12], which is an activator of the nuclear factor-κB (NFκB) transcription factor. Proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), are promoted by this factor [13]. In cisplatin renal injury, the production of TNF-α mRNA is increased [14].

The nuclear factor (erythroid derived 2)-related factor 2 (Nrf2) transcription factor is responsible for regulating the gene expression of phase II detoxification enzymes and antioxidant proteins [15]. Both Nrf2 and NFκB are regulated by redox sensitive factors and the absence of Nrf2 is associated with amplification of cytokine production, as NFκB is more readily activated in oxidative environments [16]. The anti-inflammatory role of Nrf2 is well established [17]. The modulation of Nrf2 in response to NFκB activation can act as a protective mechanism against the consequences of inflammation [18].

Upregulation of p53 protein is one of the important cellular responses to DNA damage caused by cisplatin in different cells, which is usually in normal cells is undetectable [19,20]. Activated p53 interacts with specific DNA sequences that promote the transcription of certain genes which include genes related to cell cycle arrest and apoptosis, [20,21] in a cell- and tissue-specific manner [22]. The p53 up-regulated modulator of apoptosis (PUMA) is strong proapoptotic particle which is quickly expressed in cells subsequently to the DNA damage also it was recognized as a main downstream mediator of the killing action of p53 [23].

To curtail these side effects various approaches have been attempted for years. Novel renoprotective interventions can be developed depending on the understanding of the mechanism of cisplatin nephrotoxicity. Biochanin A ($C_{16}H_{12}O_5$) is a phytoestrogen and a major active ingredient of *Astragalus membranaceus*. Biochanin A is an *O*-methylated isoflavone. 5,7-dihydroxy-3-(4-methoxyphenyl) chromen-4-one is the chemical name of biochanin A [24]. It is pharmacologically active phytochemical found in soy, peanuts, chickpea, alfalfa sprouts and red clover. A broad spectrum of beneficial biological effects has been reported for biochanin A, including hypolipidemic [25], antioxidative, antineoplastic [26] and anti-inflammatory [27] actions.

Furthermore, scientifically biochanin A was proven for its protection in dopaminergic neuron [28] and anticholinergic activities [29]. The cognitive performance in postmenopausal women has been traditionally improved by an edible bean, Soy [24]. Soy also used to halt the degenerative process in the central nervous system and progression of Alzheimer's disease [30]. These favorable anti-inflammatory activities led to suggesting biochanin A as a nephroprotective agent to be tested in mice against cisplatin nephrotoxicity. The effect of biochanin A on renal function, histopathology and tubular apoptosis will be determined to achieve that goal.

## 2. Materials and methods

### 2.1. Animals

Experiments were carried out on thirty-six male albino Swiss mice, weighing (20 ± 5 g). Mice were purchased from the Egyptian Organization for Biological Products and Vaccines. Animals were kept in the animal house of Faculty of Pharmacy, Suez Canal University, under environmentally controlled conditions (temperature 25 ± 4 °C and normal light/dark cycle) with free access to standard food and water *ad libitum*. They were kept for a week of acclimatization before starting the experiment. Experiments were conducted after approval from the Institutional Research Ethics Committee (Approval Number: 201512A5). This study was conducted in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

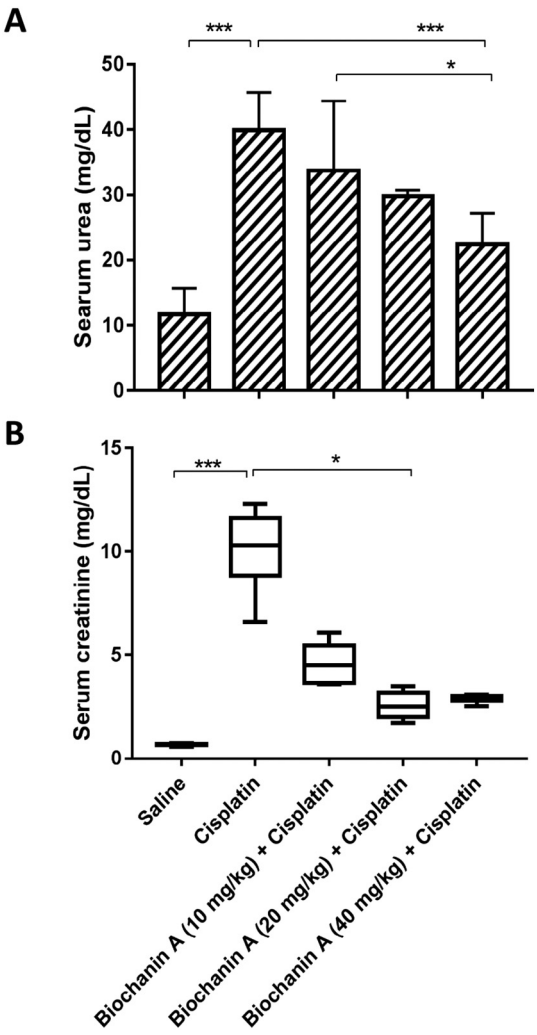


**Fig. 1.** Serum level of creatinine and urea in the experimental groups. Serum urea (A) and creatinine (B). Biochanin A was given by intraperitoneal injection for 14 days and then mice were injected with an acute dose of cisplatin (10 mg/kg). Data for serum urea are mean ± SD and one-way ANOVA was applied followed by Bonferroni's post-hoc test. Data for serum creatinine are medians and quartiles and were analyzed using the Kruskal-Wallis non-parametric ANOVA followed by Dunn's test. $^{*}P$ value $< 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

### 2.2. Drugs and chemicals

Biochanin A was purchased from Sigma-Aldrich (MO, USA). A stock solution was prepared first in DMSO, then a freshly-prepared working solution was obtained by mixing 0.7 mL of the stock solution with 6.3 mL of phosphate-buffered saline (1:9, DMSO: PBS). Cisplatin vial (10 mg/10 mL) was purchased from Oncotec Pharma Produktion GmbH AM (Pharmapark 06861 Dessau-Roblau, Germany). Dimethylsulfoxide (DMSO) and alcohol were of analytical grade and purchased from commercial sources.

### 2.3. Design of the experiment

Mice were divided into 6 groups and each group comprising of six animals.

**Group I**: mice were injected daily with the vehicle of biochanin A (1:9 mixture of DMSO and saline, 0.2 mL/mouse, i.p.) and served as a saline control group.

**Group II**: mice were injected with 0.2 mL of the vehicle (1:9 DMSO/PBS mixture) for 14 days. This group served as cisplatin control group.

**Groups III**, **IV** and **V**: mice were injected with biochanin A (10, 20