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# Protective effects of zingerone on oxidative stress and inflammation in cisplatin-induced rat nephrotoxicity

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ABSTRACT

Cisplatin is one of the most commonly used and highly effective cancer chemotherapeutic agents. Use of cisplatin is limited due to persistence of severe side effects such as nephrotoxicity, neurotoxicity, and hearing loss. Nephrotoxicity is the most common limiting side effect of cisplatin use. Zingerone is one of the active ingredients present in ginger plant that has anti-inflammatory and antioxidant effects. In this study, Wistar rats were assigned randomly to 6 groups with 5 animals in each group. The control group; cisplatin group which received 7.5 mg/kg of cisplatin intraperitoneally (i.p.) at the 4th day; zingerone group received 50 mg/kg of zingerone orally for 7 days. Three other groups were pretreated with 10, 20, and 50 mg/kg of zingerone orally for 7 days and cisplatin administered 7.5 mg/kg i.p. at the 4th day, respectively. The animals were sacrificed 72 h after cisplatin injection and blood samples were taken to evaluate the serum factors. Right kidneys were collected for histopathological studies and left kidneys were considered to measure the oxidative stress parameters and TNF-α cytokine. Co-administration of zingerone along with cisplatin resulted a statistically significant reduction in lactate dehydrogenase (LDH) activity, creatinine and BUN levels of serum in comparison with cisplatin alone group ($P < 0.01$). Zingerone significantly decreased the tissue levels of malondialdehyde (MDA) ($P < 0.05$) and significantly retained the enzyme activity of catalase (CAT) ($P < 0.05$) and glutathione peroxidase (GPX) ($P < 0.05$) in kidney tissue compared to cisplatin. Zingerone did not permit the reduction of glutathione (GSH) levels ($P < 0.001$) in kidney tissue and by reducing the level of tumor necrosis factor (TNF)-α ($P < 0.05$) suppressed the inflammation produced by cisplatin. Furthermore, zingerone improved histopathological changes such as vacuolation (fat deposit), brush border loss, infiltration of leukocytes, glomerular diameters and congestion of RBCs. However, our findings suggest that zingerone has nephroprotective effects in cisplatin rat model of nephrotoxicity mostly through suppression of oxidative stress and inflammation.

## 1. Introduction

Cisplatin or cis- dichlorodiaminoplatinum (II) is a widely used anticancer drug [1]. Cisplatin is used in treatment of testicular [2], bladder [3], ovary [4], lung [5], and head and neck cancers [6]. It has been reported that 30% of patients treated with cisplatin suffer from nephrotoxicity [7]. Cisplatin use is associated with several side effects such as nephrotoxicity, severe nausea and vomiting, bone marrow suppression, ear toxicity (hearing loss), neurotoxicity and allergic reactions [8]. Nephrotoxicity is the main limiting toxicity of cisplatin use and is dose dependant [9]. Nephrotoxicity occurs in 20% of patients following the first dose of cisplatin [10]. Nephrotoxicity is characterized by reduction in glomerular filtration, increase of serum creatinine and BUN levels, hypokalemia, and hypomagnesemia [11]. Cisplatin is mainly accumulated in proximal tubules [12]. Most damaged caused by cisplatin occurs in proximal tubules, especially S3 region, followed by glomeruli and distal tubules [13]. Various mechanisms have been reported for pathogenesis of cisplatin nephrotoxicity which include production of nephrotoxic metabolites, oxidative stress, vascular injury, inflammation, production of free radicals, and activation of apoptotic

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pathways [14]. Administration of cisplatin result in production of hydroxyl, super oxide, and hydrogen peroxide radicals in kidney tissues. These radicals react with various cellular components such as DNA, protein, and membrane lipids [15]. Recently, it has been reported that inflammation plays an important role in cisplatin nephrotoxicity [16] where, TNF-α cytokine plays the most crucial role [17].

The main strategy for prevention of cisplatin nephrotoxicity is use of normal saline followed by administration of diuretics [18]. Studies have revealed that use of antioxidants and free radical neutralizing agents such as vitamin E [19], lipoic acid [20], and *N*-acetylcysteine [21] could be useful in reduction of cisplatin associated nephrotoxicity [22].

Ginger (*Zingiber officinale*) belongs to zingiberacea family and is composed of active ingredient such as zingerone, gingerol, paradol, and shogaol [23]. It has been shown that zingerone with chemical formula 4-(4-Hydroxy-3-methoxyphenyl) butan-2-one) has protective effects in rat animal models of liver injuries [24]. It is a pharmacologically active ingredient of ginger that is present in dried ginger and is converted from zingerol to zingerone while drying or boiling. Moreover, the contents of gingerol which is another component in ginger, including 6-gingerol, 8-gingerol, and 10-gingerol are commonly low in fresh ginger while on drying and roasting the amount of zingerone increases significantly. Zingerone known as vinillyl acetone, shown anti-angiogenic effects [25]. Zingerone is reported to have therapeutic effects such as antioxidant, anti-inflammatory, anti-obesity, oxidative stress antagonist, anti-nausea and anti-vomiting agent after chemotherapy, and anti-diuretic agent [26].

Previously, it has been reported that aqueous extract of zinger is capable of producing protective effects against cadmium associated nephrotoxicity [27]. It has also been shown that 6-gingerol and alcoholic extract of ginger have potential protective activity against cisplatin induced nephrotoxicity [28,29]. Thus, zingerone should have potential protective effects against cisplatin induced nephrotoxicity as zingerone is one of the active components of ginger.

## 2. Materials and methods

### 2.1. Chemicals and kits

Zingerone was purchased from Sigma Aldrich; cisplatin vial was obtained from Hospira, UK. The used kits concluding catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPX) were purchased from ZellBio Company. TNF-α cytokine (abm 10,075) kit was purchased from Abcam Company.

### 2.2. Animals

In this experimental study, male Wistar rats were used at six weeks of age and weighing 170–200 g. Animals were purchased from the Animal Center of Ahvaz Jundishapur University of Medical Science (Ahvaz, Iran). The study was approved by Ethical Committee Acts of Ahvaz Jundishapur University of Medical Science for care and use of laboratory animals (IR.AJUMS.REC.1396.33). The rats were allowed to acclimate to their surrounding environment for 1 week prior to the start of the experiments. Five rats were housed per polypropylene cage and kept at 25 ± 2 °C, 12 h light/dark cycle and given standard rat chow and drinking water ad libitum.

### 2.3. Experimental groups

To study the effects of zingerone on oxidative stress and inflammation in cisplatin induced kidney damage, 30 rats were randomly assigned to 6 groups.

I Control group (vehicle) received 5 ml/kg of normal saline orally for 7 days.
II Cisplatin group received 7.5 mg/kg [30] of cisplatin i.p. at the 4th day.
III Zingerone group received 50 mg/kg of zingerone orally for 7 days.
IV Zingerone 10 + cisplatin group received zingerone 10 mg/kg orally for 7 days and cisplatin 7.5 mg/kg of i.p. at the 4th day.
V Zingerone 20 + cisplatin group received zingerone 20 mg/kg orally for 7 days and cisplatin 7.5 mg/kg i.p at the 4th day.
VI Zingerone 50 + cisplatin group received zingerone 50 mg/kg orally for 7 days and received cisplatin 7.5 mg/kg i.p at the 4th day [31,32].

The solvent of both cisplatin and zingerone was normal saline. 72 h after administration of cisplatin, all animals were anaesthetized by ketamine (100 mg/kg) and xylazine (10 mg/kg) and blood samples were collected from carotid artery. Blood samples were centrifuged at 3000 rpm for 10 min to separate serum. Kidneys were removed and weighed immediately. Right kidneys were considered for histopathological studies and they were kept at 10% formalin buffer at pH = 7.4. Left kidneys were kept at −70 °C to be used later for oxidative stress assessment and TNF-α measurement.

### 2.4. Homogenization

Kidney tissues were homogenized 1:10 with phosphate buffer saline (pH = 7.4) and centrifuged at 12,000 rpm for 10 min. The supernatant was collected and used to measure level of TNF-α cytokine and also for oxidative stress testing including enzymatic activity of CAT, SOD, and GPX and measurement of GSH and MDA in kidney tissue.

### 2.5. Determination of body weight change and relative kidney weight

Animals were weighed on day 1 and day 7 of experiment and the weight changes were calculated based on initial weight. To determine the kidney index percentage, average kidney weight of each animal was divided by weight of animal at day 7 and the value was represented at percentage [33].

### 2.6. Determination of serum factors

Kidney function indicators including blood urea nitrogen (BUN), albumin, creatinine, and lactate dehydrogenase (LDH) activity were measured using standard diagnostic kits (Pars Azmoon Kit. IRI) and clinical spectrophotometer (UV-1650 PC, Shimadzu, Japan).

### 2.7. Oxidative stress testing

Measurement of MDA and GSH levels and enzyme activities of CAT, SOD and GPX were done according to the standard protocols provided from ZellBio Company.

### 2.8. Measurement of TNF-α cytokine

The amount of TNF-α in kidney tissue was determined using Enzyme-linked immunosorbent assay (ELISA) kit technique using provided protocols from Abcam Company.

### 2.9. Histopathological changes

For each rat, 6 microscopy of H&E (Hematoxylin and Eosin) stained sections were assessed for histological criteria including nuclear proximal cell vacuolation, accumulation of inflammatory cells, brush border loss and congestion of red blood cells (RBC). The average percentage of each feature was determined. Infiltration of inflammatory cells and congestion of RBCs were graded into 4 categories: normal (0), weak (1), moderate (2) or intense (3) and the averages were considered. Glomerular diameter was measured by using Motic Images Plus 2.0, an