Food and Chemical Toxicology 81 (2015) 120–128

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Vetiver oil (Java) attenuates cisplatin-induced oxidative stress, nephrotoxicity and myelosuppression in Swiss albino mice

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ARTICLE INFO

Article history:
Received 7 January 2015
Accepted 15 April 2015
Available online 21 April 2015

Keywords:
Cisplatin
Vetiver acetate oil
Oxidative stress
Nephrotoxicity
Genotoxicity
Myelosuppression

ABSTRACT

Clinical efficacy of the widely used anticancer drug cisplatin is limited due to its adverse side effects in normal tissues mediated by oxidative stress. This study was aimed to investigate the protective effects of vetiver acetate oil, Java (VO) against cisplatin-induced toxicity in Swiss albino mice. The ameliorating potential was evaluated by orally priming the animals with VO at doses 5, 10 or 20 mg/kg bw for 7 days prior to cisplatin treatment. Acute toxicity in mice was induced by injecting cisplatin (3 mg/kg bw) intraperitoneally for 5 consecutive days. Significant attenuation of renal toxicity was confirmed by histopathological examination, lowered levels of serum blood urea nitrogen, creatinine and reduced DNA damage. VO also compensated deficits in the renal antioxidant system. VO intervention significantly inhibited DNA damage, clastogenic effects, and cell cycle arrest in the bone marrow cells of mice. Hematological parameters indicated attenuation of cisplatin-induced myelosuppression. Overall, this study provides for the first time that VO has a protective role in the abatement of cisplatin-induced toxicity in mice which may be attributed to its antioxidant activity.

1. Introduction

Cisplatin emerged as one of the principal chemotherapeutic agents to treat various human malignancies (Fram, 1992). The extensive application of cisplatin in clinical oncology, particularly high dose and repeated cycles of treatment regimen has been limited by its undesirable side effects. Cancer patients receiving cisplatin are at high risk of drug-induced renal toxicity with varying renal tolerance profiles (Chirino and Pedraza-Chaverri, 2009; Yao et al., 2007). The mechanism of cellular damage induced by cisplatin is a complex process involving oxidative stress, apoptosis, inflammation and fibrogenesis (Lee et al., 2006; Pabla and Dong, 2008). Cisplatin induces renal toxicity by accumulating cisplatin in tubular cells (Arany and Safirstein, 2003). Additionally, clastogenic activity of cisplatin in non-tumor cells and cumulative myelosuppression often leads to anemia intensifying the complications of chemotherapy (Attia, 2010; Ghosh et al., 2013; Hoagland, 1982).

Despite understanding the mechanism of cisplatin-induced toxicity, prevention still relies on decrease in drug dosage and hydration of patients (Yao et al., 2007). To date, numerous compounds have been screened to ameliorate cisplatin-induced toxicity. Amifostine, a synthetic antioxidant drug is the only FDA approved therapeutic agent for the reduction of cisplatin-induced renal toxicity (Capizzi, 1999). However, use of amifostine is limited by its side effects, cost, and concerns about possible interference with the anti-tumor activity of cisplatin. On the contrary natural antioxidants have been known to impart protection without compromising the anti-tumor potential of chemotherapeutic drugs and side effects (Orsolic and Car, 2014; Orsolic et al., 2013; Sanchez-Gonzalez et al., 2011). Therefore, studies on screening of potential phytochemicals to enhance the efficacy of chemotherapeutic agents and reduce its undesirable side effects are of great interest.

Essential oil of vetiver (VO), extracted from the roots of the medicinal and aromatic plant *Chrysopogon zizanioides* (L.) Roberty or *Vetiveria zizanioides* (L.) Nash is well known for its ethnobotanical relevance and traditional uses (Anonymous, 1976). Recently, various beneficial and therapeutic properties of VO such as hepatoprotective (Parmar et al., 2008, 2013), anti-hyperglycemic (Karan et al., 2012; Rajeswari and Rajagopalan, 2013), anti-inflammatory (Lima et al., 2012) and antioxidant properties (Fazal et al., 2011; Kim et al., 2005; Subhadradevi et al., 2010) have been established in *in vitro* and *in vivo* models. Studies by Kim et al. (2005) showed strong free radical scavenging activity of vetiver oil (VO) when compared with the standard antioxidants such as butylated hydroxytoluene (BHT) and

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http://dx.doi.org/10.1016/j.fct.2015.04.018
0278-6915/

alpha-tocopherol. VO exhibited ~93% free radical scavenging activity in the DPPH assay and in contrast, BHT and alpha-tocopherol exhibited ~93 and 89% free radical scavenging activities respectively.

Considering the potent antioxidant activity of vetiver oil (VO), the present study investigated its ameliorating efficacy against cisplatin-induced nephrotoxicity and myelosuppression in Swiss albino mice. Evaluation of renal histopathology, serum blood urea nitrogen, creatinine, renal protein carbonyls, malondialdehyde, reduced glutathione (GSH), glutathione-S-transferase (GST) and DNA damage was performed to assess nephrotoxicity. Bone marrow toxicity was analyzed by chromosomal aberration test, micronuclei formation, comet assay, and cell cycle progression. In addition, hematology profile was also determined.

2. Materials and methods

2.1. Test substances

Vetiver acetate, Java (CAS-No. 62563-80-8) and cis-Diammineplatinum (II) dichloride (cisplatin, CAS-No. 15663-27-1) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and used in the present study.

2.2. Animal handling and care

Healthy male Swiss albino mice (8–12 weeks old and weighing 25–30 g) were maintained under standard laboratory conditions [14 h: 10 h dark/light cycle, a temperature of (22 ± 2 °C), and 50–70% humidity]. The animals were fed on standard rodent pellets and drinking water *ad libitum*. Our investigation conforms to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and also approved by the Institutional Animal Ethics Committee (IEAC), University of Calcutta (Registration #885/ac/05/CPCSEA), registered under "Committee for the Purpose of Control and Supervision of Experiments on Laboratory Animals" (CPCSEA), Ministry of Environment and Forests, Government of India.

2.3. Effect of VO on Swiss albino mice

2.3.1. Dose selection and treatment schedule

Vetiver oil (Java) has a reported LD50 value of >5000 mg/kg in rabbit and rat models (Opdyke, 2013). In addition, more recent publication by Tripathi et al. (2006) reported of a much lower LD50 value of 2985 mg/kg bw from their acute exposure experiments in mice. In light of the above mentioned studies, exposure concentrations of VO for the present study was set below 1/100th of the reported LD50 value in mice (i.e., 2985 mg/kg bw). To evaluate the effect of VO, mice were randomly divided into four groups with five animals in each. The control group animals received 0.1% (v/v) of Tween-20 in double distilled water by gavage for 7 days. Animals belonging to the other three groups were gavaged with VO for 7 days at different doses of 5, 10 or 20 mg/kg bw respectively and evaluated for possible toxic effects. The concentration of Tween-20 was 0.1% (v/v) in the highest treatment dose of 20 mg/kg bw. After sacrificing the animals by cervical dislocation, biochemical assays such as lipid peroxidation, reduced glutathione (GSH) and Glutathione-S-transferase (GST) activity and DNA damage analysis by alkaline comet assay was performed.

2.3.2. Biochemical assays

After sacrificing the mice, the kidneys were excised, washed and blotted dry. Lipid peroxidation level in kidney homogenates was quantified spectrophotometrically at 535 nm according to Buege and Aust (1978). Reduced glutathione (GSH) level was estimated by the method of Sedlak and Lindsay (1968) and GST activity was measured according to the method of Habig et al. (1974). Protein quantification in kidney homogenate was carried out spectrophotometrically by the method of Bradford (1976) with bovine serum albumin as a standard.

2.3.3. DNA damage in renal and bone marrow cells

DNA damage was evaluated using alkaline comet assay according to the method of Singh et al. (1988) with slight modifications. Animals were sacrificed, kidneys and femurs were excised and single cell suspension was prepared in phosphate buffered saline (Manivannan et al., 2013; Sasaki et al., 2002). Briefly, kidneys were minced and femurs were flushed in physiological saline containing 0.024 M Na_2EDTA (pH 7.5). Followed by centrifugation, the cells were resuspended in phosphate buffered saline. Slides were prepared by mixing the cell suspension with 1% low melting point agarose; layered on the slide base coated with 1% normal melting point agarose and placed in chilled lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Trizma, 10% DMSO, and 1% Triton X-100, pH 10.0) at 4 °C for 1 h. Then the slides were subjected to DNA unwinding in chilled alkaline solution (300 mM NaOH and 1 mM Na_2EDTA, pH >13) for 20 min and subsequently electrophoresis was performed at 0.7 V/cm and 300 mA at 4 °C for 30 min in freshly prepared electrophoresis buffer (300 mM NaOH and 1 mM Na_2EDTA, pH >13). After electrophoresis the slides were neutralized with Tris buffer (400 mM, pH 7.4). Slides were stained with ethidium bromide (20 μg/ml) and cells were scored using a fluorescence microscope. A computerized image analysis system (Komet version 5.5, Kinetic Imaging Ltd., Andor Technology, Nottingham, UK) was employed. Tail intensity (DNA percentage in the comet) was used to measure DNA damage.

2.4. Evaluation of the ameliorating potential of VO against cisplatin-induced toxicity

2.4.1. Treatment schedule

Prior to cisplatin administration, mice were gavaged with VO for 7 days at different doses of 5, 10 or 20 mg/kg bw and evaluated for possible mitigation of cisplatin-induced toxicity. Cisplatin was injected to mice intraperitoneally at 3 mg/kg bw (equivalent to 9 mg/m^2) for 5 consecutive days and were sacrificed after 5 days. Cisplatin at 3 mg/kg bw exhibited significant toxicity without compromising the survival of the animals. Cisplatin was reconstituted in 0.9% NaCl and used within 1 h of preparation. Experimental animals were randomly divided into 5 treatment groups (I–V) with five animals in each.

- Group I (Vehicle control) – Animals received 0.1% (v/v) of Tween-20 in double distilled water by gavage for 7 days.
- Group II (Cisplatin control) – Animals received 0.1% (v/v) of Tween-20 in double distilled water by gavage for 7 days. From day 7 to 11, cisplatin was injected intraperitoneally at 3 mg/kg bw for 5 days consecutively.
- Group III (Cis+VO, 5 mg/kg) – Animals received VO by oral administration at dose of 5 mg/kg bw, for 7 days. From day 7 to 11, cisplatin was injected intraperitoneally at 3 mg/kg bw for 5 days consecutively.
- Group IV (Cis+VO, 10 mg/kg) – Animals received VO by gavage at 10 mg/kg bw, for 7 days. From day 7 to 11, cisplatin was injected intraperitoneally at 3 mg/kg for 5 days consecutively.
- Group V (Cis+VO, 20 mg/kg) – Animals received VO by oral administration 20 mg/kg bw, for 7 days. From day 7 to 11, cisplatin was injected intraperitoneally at 3 mg/kg bw for 5 days consecutively.

The animals were sacrificed on day 16 and various end point assays were performed after removal of the kidneys and femoral bones. Before sacrificing the animals by cervical dislocation, blood samples were collected into EDTA vacutainers via cardiac puncture under isoflurane anesthesia. Histopathology, comet assay, micronucleus test and cell cycle analysis were conducted in the same set of animals, whereas the enzyme assays and chromosome aberration test was performed in another set of animals having identical treatment groups.

2.4.2. Change in bodyweight and reno-somatic index

The bodyweight of each animal was recorded before treatment and on day 16 of the experiment. Percentage change in bodyweight of mice was calculated. After mice were sacrificed, kidneys from the animals were quickly excised. The kidneys were cleaned, blotted, and weighed, and the ratio between the kidney weight and bodyweight of the animals were determined. Data of reno-somatic index are expressed as a ratio of kidney weight over bodyweight (Manivannan et al., 2013).

2.4.3. Histopathology of kidney tissue

Kidneys were excised from mice, washed, and fixed in 10% neutral buffered formalin. The tissue sections (5 μm thick) were prepared from paraffin-embedded tissues and stained with hematoxylin and eosin. Stained tissue sections were observed under a light microscope for changes in organizational structure for histological evaluation.

2.4.4. Estimation of serum blood urea nitrogen (BUN) and creatinine levels

Blood samples collected from mice were centrifuged to isolate the serum. Serum BUN and creatinine levels were quantified spectrophotometrically by standard enzymatic methods (Carl Allinson, 1945; Mather and Roland, 1969) using commercially available kits (Span Diagnostic Pvt. Ltd, Surat, India).

2.4.5. Assessment of oxidative stress in kidney

Protein oxidation was quantified as protein-bound carbonyl content in the kidney homogenate by its reaction with 2, 4-dinitrophenylhydrazine (DNPH) giving a yellow colored hydrazone quantified spectrophotometrically at 340 nm (Levine et al., 2000). Lipid peroxidation, reduced glutathione (GSH) level and GST activity were measured as described previously under section 2.3.2.

2.4.6. Genotoxicity evaluation

2.4.6.1. DNA damage in renal and bone marrow cells. DNA damage was analyzed by alkaline comet assay as described previously under section 2.3.3.

2.4.6.2. Micronucleus assay (MN). Micronucleus test was carried out in femoral bone marrow cells according to the method of Schmid (1976). The slides were prepared and stained with May–Gruenwald–Giemsa and observed under light microscope (Carl, Zeiss, Berlin, Germany). All slides were coded blind and scored. The incidence of micronucleated (MN) cells per 500 polychromatic erythrocytes (PCEs) was counted for each animal and the percentage of PCEs with MN was calculated.