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Protective role of *Emblica officinalis* hydro-ethanolic leaf extract in cisplatin induced nephrotoxicity in Rats

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ABSTRACT

Nephrotoxicity is a major limiting factor in cisplatin treatment. In the present study hydro-ethanolic leaf extract of *Emblica officinalis* was investigated for its protective role in cisplatin induced nephrotoxicity. The experiment was designed for 14 days and male Wistar rats were divided into 9 groups (n = 5). Group 1 served as control (with no treatment), group 2 served as a vehicle control and received 0.9% NaCl intraperitoneally (i.p.) on 11th day of the treatment, group 3 received a single dose of cisplatin on 11th day (12 mg/kg body weight, i.p.), group 4–6 received leaf extract only (100 mg/kg, 200 mg/kg and 400 mg/kg body weight, respectively) throughout the treatment, group 7–9 received leaf extract (100 mg/kg, 200 mg/kg and 400 mg/kg body weight, respectively) throughout the treatment and single dose of cisplatin on the 11th day of the leaf extract treatment. At the end of the experiment (*i.e.* on 14th day) blood samples were collected from all the groups and were sacrificed to study renal functional parameters. Treatment with above doses of *E. officinalis* leaf extract significantly ($p \leq 0.05$) attenuates renal damage by decreasing serum creatinine and blood urea nitrogen (BUN), enhanced the activities of Catalase, SOD, GPx, GR and decreased the renal MDA level compared with the cisplatin treatment group. Furthermore the oral administration of Amla leaf extract improves histological damage and morphological changes in RBCs. Our results suggest that, leaf extract of *E. officinalis* may ameliorate renal damage caused by cisplatin.

1. Introduction

Cisplatin (Cis-diamminedichloro platinum II) also called as "The Penicillin of cancer" was the first big chemotherapy drug [1]. FDA in 1978, approved cisplatin as leading anti- cancer drug for several types of cancer, such as bladder cancer, cervical cancer, ovarian cancer, testicular cancer, non-small cell lung cancer, squamous cell carcinoma of the head & neck, with 90% cure rates in testicular cancer, either used alone or combined with other drugs. Even with the advancement of new therapies in the past decades, the use of cisplatin remains strong [2–4]. Although the profound effects of cisplatin in different types of the cancer, the patients experience severe side effects such as vomiting and nausea, myelosuppression, neurotoxicity, ototoxicity and nephrotoxicity that limit its use [5]. Nephrotoxicity is the major limitation in cisplatin treatment as Kidney is exposed to large amounts of parent and active metabolites of drugs. The toxic effects of cisplatin in kidney affects approximately 25–35% of patients treated with a single dose, thereby limiting its use in higher doses and compromising its chemotherapeutic efficacy [4]. In kidney the proximal tubules especially S3 segment is severely affected by the cisplatin treatment and is characterized by the loss of microvilli, cellular swelling and condensation of nuclear chromatin. Other functional characters of renal dysfunction are decrease in glomerular filtration, increased serum creatinine and BUN levels, increased lipid peroxidation, and decrease in enzymatic and non enzymatic antioxidants [2,4,6].

Cisplatin causes tubular cell death by either necrosis or apoptosis depending upon the concentration and duration of exposure of drug. Many of the pathways involved in tumor cell death involve mitochondrial dysfunction and oxidative stress. Many studies have focused on the use of natural and synthetic antioxidants or ROS scavengers for Reno-protection [7]. *In vitro* and *in vivo* studies have reported protective effect of several natural and synthetic antioxidants in cisplatin induced

Abbreviations: BUN, blood urea nitrogen; BW, body weight; BSA, bovine serum albumin; CAT, catalase; DNA, deoxy-ribonucleic acid; DMSO, dimethyl sulfoxide; DTNB, 5,5-dithio-bis(2-nitrobenzoic acid); FDA, food and drug administration; GPx, glutathione peroxidase; GR, glutathione reductase; GAMT, guanidinoacetate methyltransferase; H&E, hematoxylene and eosin; MDA, malondialdehyde; NaCl, sodium chloride; NADPH, nicotinamide adenine dinucleotide phosphate; RBCs, red blood cells; ROS, reactive oxygen species; RNS, reactive nitrogen species; SOD, superoxide dismutase; SEM, standard error mean; TBA, 2-thiobarbituric acid; TCA, trichloroacetic acid

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nephrotoxicity [8–10]. However, lack of data regarding their effects on the antitumor activity of cisplatin and lack of clinical trials limits the clinical application of compounds [4,11,12].

E. officinalis commonly called as Amla (Indian gooseberry) is one of the most studied plant. Plant parts of *E. officinalis* show antibacterial, antioxidant, antidiabetic, hypolipidemic, anticancer, hepatoprotective, gastroprotective, antiulcerogenic, nephroprotective and chemopreventive properties [13,14]. Most important part of plant is fruit and has been explored more for its medicinal values. However, the leaves are explored substantially less. The active constituents of leaves include Apigenin-7-*O*-(6_-butyryl- _-glucopyranoside), flavanone glycosides, gallic acid, methyl gallate, luteolin-4_-O-neohesperidoside, 1,2,3,4,6-penta-*O*-galloylglucose, trihydroxysitosterol, 5 _, 6 _, 7 _-acetoxysitosterol, 5-hydroxymethylfurfural, 2-acetyl-5-methyl furan, pyrogallol, ellagic acid [15–18]. The active constituents of leaves of *E. officinalis* show antibacterial and antioxidant properties [19], anti-inflammatory properties [20,21]. Huang and Zhong [22] studied the anticancer mechanism of gallic acid isolated from *Phyllanthus emblica* against hepatocellular carcinoma cells. Nain et al. [23] reported antidiabetic and antioxidant properties of leaves against streptozotocin-induced type-2 diabetes mellitus (T2DM) rats by enhancing the activities of antioxidant enzymes Recently, Malik et al. [24] reported *E. officinalis* dried fruit extract ameliorates cisplatin induced renal damage through suppression of MAPK induced inflammation and apoptosis.

Based on the pharmacological and ethno botanical reports of *E. officinalis*, the present investigation was focused on studying antioxidant properties of plant and protective role of its hydro-ethanolic leaf extract in cisplatin induced nephrotoxicity. We measured enzymatic activities such as SOD, CAT, GPx, GR, along with the hematological and histopathological parameters.

2. Materials and method

2.1. Chemicals

Cisplatin was purchased from Thermo Fisher, Waltham, USA. Sulphanilamide, EDTA (Ethylenediamine- tetra acetic acid disodium salt dihydrate), NBT (Nitroblue tetrazolium chloride), Methionine, Riboflavin were procured from Himedia, Mumbai, India. Creatinine assay kit (Erba) was purchased from Transasia Bio-medicals Ltd., Solan, H.P. India, and Urea assay Kit (Auto span) was purchased from Arkray healthcare Pvt. Ltd., Gujrat, India, Hydrogen peroxide was obtained from Fisher Scientific Mumbai, India. All other chemicals were of analytical grade and were purchased from local vendors supplying scientific grade chemicals.

2.2. Preparation of leaf extract

The leaves of *Emblica officinalis* (Amla) were collected from areas near Bilaspur district. The leaves were identified and authenticated by qualified taxonomist from Guru Ghasidas University, Bilaspur, C.G. The collected leaves were washed with tap water to remove dirt and air dried at room temperature for 7 days. The dried leaves were grounded into powder form; 30 g of grounded leaves was extracted by 70% ethanolic solvent using soxhlet extractor for 6–8 h at 70 °C. After the extraction, the extracts were concentrated by evaporating the solvent until it got reduced to a solid mass.

2.3. Animals

Male Wistar rats weighing between 120 to 180gm were used in the experiment. Animals were maintained under controlled environment. Five animals per cage were placed at a temperature of 22 ± 2 °C with 12 h light and 12 h dark cycle. Rats were kept on bed of rice husk and fed on standard rodent diet and water *ad libitium*.

All animal experiments were performed subsequent to the approval of institutional animal ethical committee and by animal regulatory body of the government (Regd. No. 1321/PO/ReBi/10/CPCSEA).

2.4. Experimental design

Male Wistar rats were divided into nine groups each group containing five rats. Total duration of experiment was 14 days. Group 1 served as control (with no treatment), group 2 served as vehicle control and given vehicle only (0.9% saline) on 11th day of the treatment, group 3 received a single dose of cisplatin (12 mg/kg body weight) in 0.9% saline on 11th day, groups 4–6 were orally administered hydro ethanolic extract of *Emblica officinalis* leaves (100 mg/kg, 200 mg/kg and 400 mg/kg body weight, respectively) during entire treatment period, groups 7–9 received hydro ethanolic extract of *Emblica officinalis* leaves (100 mg/kg, 200 mg/kg and 400 mg/kg body weight, respectively) during entire treatment period and along with a single dose of cisplatin (12 mg/kg body weight) in 0.9% saline on 11th day.

Cisplatin was prepared in normal saline (0.9% NaCl) and administered intraperitoneally (i.p). Plant extracts were administered *via* oral gavage and prepared in normal water. Dose of Amla leave extract was selected on the basis of acute toxicity study in its leaves extract [23]. At the end of the experiment (*i.e.* 14th day of experiment) blood samples were collected for hematological study followed by sacrificing the animal. Kidneys were dissected bilaterally one part of the kidney was fixed in Bouin's fixative for histopathological study and the other part was kept frozen in −80 °C for enzymatic and biochemical assays.

2.5. Collection of blood and isolation of serum

Blood was collected from animals by puncturing retro-orbital venous sinus and allowed to clot at 37 °C for 40 min and centrifuged at 3000 rpm for 20 min. The serum samples was taken in a clean mini centrifuge tubes and kept refrigerated until further used.

2.6. Preparation of tissue homogenate

72 h after cisplatin treatment all rats were weighed and sacrificed for isolation of kidney. After isolation, kidney samples were immediately weighed and frozen in dry ice and stored at −80 °C until further analysis. Kidney to body weight ratio was calculated. All the enzyme assays were performed on next day of kidney isolation. The Kidney were minced into small pieces and homogenized in ice cold phosphate buffer saline (0.05 M, pH 7.0) to obtain a 10% homogenate. The homogenate was centrifuged at 17000*g* for 60 min at 4 °C and the supernatant was used for assay of protein, Catalase, SOD, GPx, GR and MDA level.

2.7. Assessment of nephroprotective activity

2.7.1. Hematological analysis

The Renal function test was determined by assaying blood urea nitrogen (BUN) and serum creatinine, BUN and creatinine concentration were measured by Urea assay Kit and Creatinine assay kit respectively.

2.7.2. Estimation of free radical scavenging enzyme

The activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GR) in kidney tissue were assayed according to the methods reported by various groups (Masayasu and Hiroshi [25], Paglia and Valentine [26], Cohen et al. [27] and Carlberg and Mannervik [28] respectively).

2.7.3. Estimation of lipid per-oxidation (LPO)

The concentration of MDA (Malondialdehyde) in kidney tissue as an index of lipid peroxidation was determined by method of Ohkawa et al. [29] using extinction coefficient of $1.56 \times 10^5\,M^{-1}\,cm^{-1}$ and expressed as n moles of MDA/g of tissue.