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Oral administration of *Nigella sativa* oil ameliorates the effect of cisplatin on membrane enzymes, carbohydrate metabolism and oxidative damage in rat liver

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ABSTRACT

Cisplatin (CP) is a potent anti-cancer drug widely used against solid tumors. However, it exhibits pronounced adverse effects including hepatotoxicity. Several strategies were attempted to prevent CP hepatotoxicity but were not found suitable for therapeutic application. *Nigella sativa* has been shown to prevent/reduce the progression of certain type of cardiovascular, kidney and liver diseases. Present study investigates whether *N. sativa* oil (NSO) can prevent CP induced hepatotoxic effects. Rats were divided into four groups viz. control, CP, NSO and CPNSO. Animals in CPNSO and NSO group were administered NSO (2 ml/kg bwt, orally) with or without single hepatotoxic dose of CP (6 mg/kg bwt, i.p.) respectively. CP hepatotoxicity was recorded by increased serum ALT and AST activities. CP treatment caused oxidant/antioxidant imbalances as reflected by increased lipid peroxidation and decreased enzymatic and non-enzymatic antioxidants. Furthermore, the activities of various carbohydrate metabolism and membrane enzymes were altered by CP treatment. In contrast, NSO administration to CP treated rats, markedly ameliorated the CP elicited deleterious alterations in liver. Histopathological observations showed extensive liver damage in CP treated animals while greatly reduced tissue injury in CPNSO group. In conclusion, NSO appears to protect CP induced hepatotoxicity by improving energy metabolism and strengthening antioxidant defense mechanism.

1. Introduction

Cisplatin (cis-diamminedichloroplatinum II, CP) is one of the most effective chemotherapeutic agent used in the treatment of variety of human malignancies including those of bladder, head, neck, testis and ovary [1,2]. However, the use of high dose of CP is difficult in clinical practice due to the associated side effects such as nephrotoxicity, neurotoxicity and ototoxicity. Recent studies suggest that hepatotoxicity is also a major dose limiting side effect in CP based chemotherapy [3,4]. High dose protocol of CP chemotherapy, therefore, necessitates the investigation of ways for prevention of dose limiting side effects that inhibit CP administration at tumoricidal doses. Until now, various methods to prevent or reduce the side effects of CP, such as synthetic antioxidants and chemoprotective agents, have been tested but effective method for clinical application has not yet been established [5,6].

CP induced toxicity is reported to be mediated by increased production of reactive oxygen species (ROS) and free radicals [3,7]. These ROS could interfere with the antioxidant defense system and can cause extensive tissue damage and cell dysfunction by reacting with macromolecules like proteins, membrane lipids and nucleic acids [7–10]. Several studies suggest that oxidative stress plays an important role in CP induced hepatotoxicity [11,12]. Naturally occurring dietary antioxidants therefore have excellent scope to protect against CP hepatotoxicity. In consequence, the search for

Abbreviations: ACPase, acid phosphatase; ALP, alkaline phosphatise; ALT, alanine aminotransferase; AST, aspartate aminotransferases; BUN, blood urea nitrogen; BBM, brush border membrane; BBMV, BBM vesicles; Scr, serum creatinine; CAT, catalase; CP, cisplatin; Chl, cholesterol; FBPase, fructose 1,6; G6Pase, glucose 6-phosphatase; G6PDH, glucose 6-phosphate dehydrogenase; GGTase, γ-glutamyl transferase; Glc, glucose; GR, glutathione reductase; GSH, glutathione; GSHPx, glutathione peroxidise; GST, glutathione S-transferase; HK, hexokinase; H_2O_2, hydrogen peroxide; LAP, leucine aminopeptidase; LDH, lactate dehydrogenase; LPO, lipid peroxidation; MDA, malondialdehyde; MDH, malate dehydrogenase; ME, malic enzyme; μm, micrometer; NADPH, nicotinamide adenine dinucleotide phosphate reduced; NADP, nicotinamide adenine dinucleotide phosphate; NSO, *Nigella sativa* oil; Pi, inorganic phosphate; PLs, phospholipids; PUFA, polyunsaturated fatty acids; ROS, reactive oxygen species; SH, sulfhydryl; SOD, superoxide dismutase; TCA, tricarboxylic acid; TR, thioredoxin reductase.

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effective, naturally occurring dietary substances with antioxidant activity, has been intensified in recent years. Plants such as *Nigella sativa* (NS) provide such a dietary source of biologically active components/phytochemicals which exhibit wide spectrum of biological properties such as antioxidant, anti-diabetic, anti-inflammatory, nephroprotective and hepatoprotective properties [13,14]. NS oil (NSO) contains fixed oil (30%) and volatile oil (0.5%), proteins, alkaloids and saponins. Thymoquinone, the principal active ingredient of NS volatile oil, possesses antioxidant potential to scavenge free radicals and to protect the cell against oxidative damage [15–17]. In addition, NSO is an important source of polyunsaturated fatty acids (PUFA) that contains omega (ω)-3 PUFA and ω-6 PUFA in the recommended optimal dietary intake ratio of 1:4 [18,19]. Consumption of ω-3 and ω-6 essential fatty acid in the right proportion has been found to suppress the pathogenesis of many diseases [20,21].

Since ancient times, NS seed/oil consumption has been shown to exhibit a multitude of beneficial health effects under normal as well as in various pathological conditions [14]. NSO has been shown to downregulate CCl_4 induced nitric oxide (NO) synthase mRNA and NO production in liver [22]. Thymoquinone, the major bioactive component of NSO, has been reported to improve plasma and liver antioxidant capacity and to enhance the expression of liver antioxidant genes in hypocholestrolemic rats [23]. Studies have shown that NSO/thymoquinone administration ameliorates CP induced nephrotoxic effects [24,25]. Thymoquinone was also found to potentiate/enhance the anti-tumor activity of CP against Ehrlich ascites carcinoma (EAC) [24]. NSO has been demonstrated to attenuate cyclosporine A and gentamicin induced renal dysfunction in experimental rats [26,27]. However, the protective potential of NSO against CP induced hepatotoxicity remains uninvestigated.

Considering the potential clinical use of CP and numerous health benefits of NSO, we hypothesized that owing to its inherent biochemical and antioxidant properties, NSO would prevent CP induced hepatotoxicity that would lead to improved metabolism and antioxidant defense mechanism of the liver.

2. Material and methods

2.1. Chemicals and drugs

N. sativa oil was supplemented from Mohammedia Products, Red Hills, Nampally, Hyderabad, India. Cisplatin was obtained from Sigma–Aldrich Chemical Corp., St. Louis, MO, USA. All other chemicals used were of analytical grade and were purchased either from Sigma Chemical Corp., or SRL (Mumbai, India).

2.2. Diet

A nutritionally adequate laboratory pellet diet was obtained from Aashirwaad Industries, Chandigarh (1544, Sector 38-B, Chandigarh, India).

2.3. Experimental design

The animal experiments were conducted according to the guidelines of Committee for purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India. Adult male Wistar rats (6 rats/group) weighing between 150 and 200 gm were used in the following experimental protocol. Rats were acclimatized to the animal facility for a week on a standard rat laboratory pellet diet and allowed water ad libitum under controlled conditions of $25 \pm 2^\circ$ C temperature, $50 \pm 15\%$ relative humidity and normal photoperiod (12 h dark and light). Four groups of rats entered the study after acclimatization; Control (C), CP treated (CP), *N. sativa* oil treated (NSO) and *N. sativa* oil + CP treated (CPNSO). Rats in the group CPNSO were administered NSO (2 ml/kg bwt, orally) for 14 days prior to and 4 days following CP treatment. However, rats in NSO group were administered NSO alone without CP treatment. CP solution (2 mg/ml) was freshly prepared in 0.9% normal saline by continuous stirring at room temperature for 10 min. A single intraperitoneal injection of CP (6 mg/kg bwt.) was administered to the animals in CP and CPNSO groups, while animals in the control and NSO group received an equivalent amount of normal saline. Body weight of rats was recorded at the start and completion of the experimental protocol. Blood was collected from fasted rats on 5th day after CP administration under light ether anesthesia. Liver was removed and processed for histopathology and the preparation of homogenate as described below.

2.4. Serum parameters

Blood samples were centrifuged at $2000 \times g$ for 10 min and sera were separated. Serum samples were deproteinated with 3% trichloroacetic acid in a ratio of 1:3, left for 10 min and then centrifuged at $2000 \times g$ for 10 min. The precipitate obtained was used to quantitate total phospholipids (PLs). Cholesterol (Chl) levels was determined directly in serum samples by method of Zlatkis et al. [28]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined in serum by Reitman and Frankel method using kit from span diagnostics.

2.5. Preparation of homogenates

Liver was homogenized in 0.1 M Tris–HCl buffer (pH 7.5) by a glass–teflon homogenizer (Thomas PA, USA) by passing 5 pulses; at 4° C to make a 10% (w/v) homogenate. The homogenate was then subjected to high-speed Ultra-Turrex Kunkel homogenizer (Type T-25, Janke & Kunkel GMBH & Co., KG. Staufen) for 3 pulses of 30 s each with an interval of 30 s between each stroke. One part of the homogenate was saved at −20° C for estimation of GSH, total-SH and lipid peroxidation while other part was centrifuged at 2000 rpm for 10 min at 4° C in high-speed Remi centrifuge (Remi motors, Mumbai, India) to remove the cell debris. The supernatant was saved in aliquots and stored at −20° C for analyses of membrane, carbohydrate metabolism and free radical scavenging enzymes.

2.6. Assay of carbohydrate metabolism enzymes

The activities of the enzymes involving oxidation of NADH or reduction of NADP were determined spectrophotometrically on UV-1700 (Pharma Spec, Shimadzu Corp., Japan) fixed for 340 nm using 3 ml of assay buffer in a 1-cm cuvette at room temperature (28–30 °C). The enzyme activities of lactate dehydrogenase (LDH), malate dehydrogenase (MDH), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G6PDH), glucose-6-phosphatase (G6Pase) and fructose-1, 6-bisphosphatase (FBPase) were assayed as described by Khundmiri et al. [29]. Hexokinase (HK) was estimated by the method of Crane and Sols [30] and the remaining glucose was measured by method of Nelson [31].

2.7. Assay of membrane enzymes and lysosomal marker enzyme

The activities of membrane marker enzymes viz. alkaline phosphatase (ALP), γ-glutamyl transferase (GGTase), leucine amino peptidase (LAP), and lysosomal marker enzyme viz. acid phosphatase (ACPase) were determined as described by Farooq et al. [32].