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Protective effect of Chlorogenic acid against methotrexate induced oxidative stress, inflammation and apoptosis in rat liver: An experimental approach

Nemat Ali, Summya Rashid, Sana Nafees, Syed Kazim Hasan, Ayaz Shahid, Ferial Majed, Sarwat Sultana*

Section of Molecular Carcinogenesis and Chemoprevention, Department of Medical Elementology and Toxicology, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India

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ABSTRACT

Methotrexate (MTX) is a drug which is used to treat different types of cancers but hepatotoxicity limits its clinical use. Chlorogenic acid (CGA) is one of the most abundant naturally occurring polyphenols in the human diet. Here, we assessed the effect of CGA against MTX-induced hepatotoxicity and investigated the underlying possible mechanisms in Wistar Rats. Rats were pre-treated with CGA (50 or 100 mg kg/b.w) and administered a single dose of MTX (20 mg/kg, b.w.). MTX caused hepatotoxicity as evidenced by significant increase in serum toxicity markers, histopathological changes. decreased activities of anti-oxidant armory (SOD, CAT, GPx, GR) and GSH content. MTX significantly causes upregulation of iNOS, Cox-2, Bax and downregulation of Bcl-2 expressions, it causes higher caspase 3, 9 activities. However CGA pretreatment alleviates the hepatotoxicity by decreasing the oxidative stress. CGA inhibited Cox-2, iNOS, Bax, Bcl-2 and Caspases 3, 9 mediated inflammation and apoptosis, and improve the histology induced by MTX. Thus, these findings demonstrated the hepatoprotective nature of CGA by attenuating the pro-inflammatory and apoptotic mediators and improving antioxidant competence in hepatic tissue. These results imply that CGA has perfective effect against MTX-induced liver injury. Hence CGA supplementation might be helpful in abrogation of MTX toxicity.

1. Introduction

Methotrexate (MTX), a folate antimetabolite, cytotoxic chemotherapeutic agent used in the treatment of different malignancies such as acute lymphoblastic leukemia, osteosarcoma, and head and neck tumors. As it is well known that chemotherapeutic agent is not selective for cancer cells but it also affects the normal cells which have high proliferation rate. Hepatotoxicity is one of the major side effects of MTX, which limits its clinical use [1,2].

Although exact mechanism of MTX induced hepatotoxicity is still unknown but several hypotheses have been given among which oxidative stress due to generation of reactive oxygen species has been well documented [3].

The imbalance between prooxidant and antioxidant defense system because of excess production of reactive oxygen species viz. superoxide radical, hydrogen peroxide, and hydroxyl radical, which pushes cell towards oxidative stress, during the oxidative stress condition the endogenous antioxidant defense mechanisms fail to protect cell from oxidative damage [4]. Oxidative stress promotes cells towards apoptosis [5].

There are several studies which suggest that the supplementation of natural compounds with antioxidant properties diminish MTX induced toxicity [3,6,7]. Therefore, compounds having antioxidant properties may boost the efficiency of chemotherapeutic drugs and also may reduce the systemic toxicity induced by chemotherapy. Drug induced liver injury (DILI) is the most frequent cause of hepatic dysfunction. Drugs or their reactive metabolites are known to induce distinct effect(s) on gene expression and cellular homeostasis in hepatocytes [8]. Chemotherapy is one of the principal methods that are employed in the management of numerous kinds of cancer, permitting the use of a combination of

* Corresponding author. Section of Molecular Carcinogenesis and Chemoprevention, Department of Medical Elementology and Toxicology, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India.
E-mail address: sarwat786@rediffmail.com (S. Sultana).

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different types of anticancer drugs to enhance its efficiency. The objective of chemotherapy is to remove specifically cancerous cells. Most of the chemotherapeutic agents act, however, non-specifically, harming both cancerous and normal cells. Toxicity of chemotherapeutic drugs to normal tissues has a significant impact on the condition and treatment outcome of patients undergoing chemotherapy. Severe side effects caused by commonly used anticancer drugs often limit the efficiency of chemotherapy [9].

Epidemiological studies indicated that diets rich in fruits and vegetables promote health and attenuate, or delay, the inception of various diseases and natural antioxidants have the potential to reduce severe side effects as well as enhance anticancer activities of antitumor drugs [10].

Chlorogenic acid (CGA), an ester of caffeic acid and quinic acid, is one of the most abundant polyphenols in the human diet, accumulating evidence has demonstrated that CGA possesses various properties such as antibacterial, anti-inflammatory and antioxidant activities, anti-inflammatory property of CGA has been related to the inhibition of nuclear factor-kappaB (NF-kB) activation and the release of pro-inflammatory cytokines in both cell cultures and mice liver [11,12]. It has been reported that CGA protects methamphetamine induced oxidative stress and liver toxicity [13]. CGA shows beneficial effects against obesity and improves lipid metabolism in high-fat diet induced obese mice [14]. Recently it has been reported that CGA protective effects against cisplatin induced kidney toxicity [15] and liver fibrosis [16].

The current study was designed to investigate the hepatoprotective effects of CGA against MTX. Probable molecular mechanisms for the therapeutic effects of CGA were investigated, including antioxidant, antiinflammatory and antiapoptotic.

2. Materials and methods

Glutathione reductase (GR), oxidized (GSSG) and reduced glutathione (GSH), 1,2-dithio-bis-nitrobenzoic acid (DTNB), 1-chloro-2, 4-dinitrobenzene, bovine serum albumin (BSA), oxidized and reduced nicotinamide adenine dinucleotide phosphate (NADP), (NADPH), chrysin, flavine adenine dinucleotide, 2,6-dichlorophenolindophenol, thiobarbituric acid (TBA), MTX etc: were obtained from Sigma-Aldrich, USA. Sodium hydroxide, ferric nitrate, trichloroacetic acid (TCA) and perchloric acid (PCA) etc were purchased from CDH, India. All other reagents used are of highest purity and commercially available.

2.1. Animals

Male Wistar rats (150–200 g), 6–8 weeks old, were obtained from the Central Animal House Facility of Hamdard University. Rats were housed in an animal care facility under room temperature (25 ± 1 °C) with 12 h light/dark cycles and were given free access to standard pellet diet and tap water. Before the treatment, rats were left for 7 days to acclimatize. Animals received humane care in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, and prior permission was sought from the Institutional Animal Ethics Committee (IAEC No: 173/CPCSEA, 28 January 2000).

2.2. Experimental procedure

Rats were randomly divided into five groups of six rats each. Group I served as control and received distilled water (vehicle of CGA) for 20 days and 0.9% saline (vehicle of MTX) on day 18 only. Group II served as toxicant and received an oral dose of distilled water daily for 20 days and a single intraperitoneal injection of MTX (20 mg/kg) on the 18th day. MTX was dissolved in 0.9% saline. Groups III and Group IV pretreated with an oral dose of CGA 50 mg/kg (D1) and 100 mg/kg (D2), respectively, for 20 days and a single intraperitoneal injection of MTX (20 mg/kg) was administered on the 18th day in both the groups. Group V received only D2 of CGA for 20 days. On 21st day the rats were sacrificed by cervical dislocation and liver samples were taken after perfusion for examination of various biochemical, immunohistochemical and histopathological parameters. Before sacrifice, rats were given mild ether anesthesia. Later on, blood was drawn from the retro-orbital sinus and serum was obtained. The excised tissue was washed with ice-cold saline (0.85% sodium chloride) and used to prepare a 10% homogenate in chilled phosphate buffer (0.1 m, pH 7.4) containing KCl (1.17%) using a Potter Elvehjen homogenizer and some tissue was also stored in 10% buffered formalin for histopathology. The dose of MTX was based on the previously reported data [17] Fig. 1.

2.3. Post mitochondrial supernatant preparation

PMS were prepared according to Tahir and Sultana with slight modification [18]. Livers was removed quickly, cleaned of extraneous material and immediately perfused with ice-cold saline (0.85% NaCl). The liver was homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%) using a Potter–Elvehjen homogenizer.The homogenate was filtered through muslin cloth and was then centrifuged at 800*g* for 5 min at 4 °C using a REMI cooling centrifuge (Cat. No. RQ127 A, Remi Motors, Mumbai, India) to separate the nuclear debris. The aliquot obtained was centrifuged at 12,000 rpm for 20 min at 4 °C to obtain PMS, which was used as a source of enzymes. All biochemical estimations were completed within 24 h of animal sacrifice.

2.4. Assay for catalase activity

The catalase activity was assessed by the method of Claiborne, 1985 [19]. In short, the reaction mixture was comprised of 0.05 ml of PMS, 1.0 ml of hydrogen peroxide (0.019 M), 1.95 ml of phosphate buffer (0.1 M, pH 7.4), in a total volume of 3 ml. Changes in absorbance were recorded at 240 nm, and the change in absorbance was calculated as nmol H2O2 consumed per min per mg of protein.

2.5. Assay for lipid peroxidation

The assay of lipid peroxidation (LPO) was done according to the method of Wright et al 1981 [20]. The reaction mixture consisted of 0.58 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml microsome, 0.2 ml of ascorbic acid (100 mM) and 0.02 ml of ferric chloride (100 mM) in a total of 1 ml. This reaction mixture was then incubated at 37 °C in a shaking water bath for 1 h. The reaction was stopped by the addition of 1 ml of TCA (10%). Following addition of 1.0 ml of TBA (0.67%), all the tubes were placed in a boiling water bath for a period of 20 min. The tubes were shifted to an ice bath and then centrifuged at 2500 × g for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm. The results were expressed as the nmol MDA formed/h/g tissue at 37 °C by using a molar extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$.

2.6. Assay for reduced glutathione

GSH was assessed by the method of Jollow et al. 1974 [21]. A quantity of 1.0 ml of 10% PMS mixed with 1.0 ml of (4%) sulphosalicylic acid was taken and then incubated at 4 °C for a minimum time period of 1 h and then centrifuged at 4 °C at 1200 × g for