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Mitigation of 5-Fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats

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ABSTRACT

5-Fluorouracil (5-FU) is a potent antineoplastic agent commonly used for the treatment of various malignancies. It has diverse adverse effects such as cardiotoxicity, nephrotoxicity and hepatotoxicity which restrict its wide and extensive clinical usage. It causes marked organ toxicity coupled with increased oxidative stress and apoptosis. Chrysin (CH), a natural flavonoid found in many plant extracts, propolis, blue passion flower. It has antioxidative and anti-cancerous properties. The present study was designed to investigate the protective effects of CH against 5-FU induced renal toxicity in wistar rats using biochemical, histopathological and immunohistochemical approaches.

Rats were subjected to prophylactic oral treatment of CH (50 and 100 mg/kg b.wt.) for 21 days against renal toxicity induced by single intraperitoneal administration of 5-FU (150 mg/kg b.wt.). The possible mechanism of 5-FU induced renal toxicity is the induction of oxidative stress; activation of apoptotic pathway by upregulation of p53, bax, caspase-3 and down regulating Bcl-2. However prophylactic treatment of CH decreased serum toxicity markers, increased anti-oxidant armory as well as regulated apoptosis in kidney. Histopathological changes further confirmed the biochemical and immunohistochemical results. Therefore, results of the present finding suggest that CH may be a useful modulator in mitigating 5-FU induced renal toxicity.

1. Introduction

5-FU is a pyrimidine antimetabolite, synthesized by Heidelberg et al. in 1957, used clinically since past 40 years. Because of its broad antitumor activity as well as its synergism with other anticancer drugs it is being used in the treatment of various types of cancers (Miura et al., 2010). The metabolic product of 5-FU is 5-fluoro-2-deoxyuridine monophosphate which is an irreversible inhibitor of thymidylate synthase, a fundamental enzyme necessary for thymine synthesis. It restrains production of deoxythymidine monophosphate (dTMP) that is crucial for replication and repair of DNA and its deficit leads to cellular toxicity (Chibber et al., 2011). Besides this, it is catabolised into dihydrouracil in liver which is cleaved into α-fluoro-β-alanine, ammonia, urea, and carbon dioxide, thereby leading to nephrotoxicity (Nora, 2012). It has also been reported to obstruct the activity of exosome complex and integrates its toxic metabolites into DNA and RNA and thus terminating cell cycle and induction of apoptosis (David et al., 2011). It is used in the treatment of various malignancies including breast, head, neck, stomach, colorectal, genitourinary tract, liver and skin cancer. 5-FU like other chemotherapeutics is non targeted in action and demolishes rapidly dividing normal cells in the patients besides tumors and results in proliferative inhibition, DNA damage and cell death leading to extensive side effects. Some of the common clinical side-effects include myelosuppression, diarrhoea, vomiting, mucositis, leukopenia, stomatitis, alopecia, cardiotoxicity, nephrotoxicty and hepatotoxicity (David et al., 2011; Lamberti et al., 2012; Chang et al., 2012; Kinhult et al., 2003 and Nora, 2012). Nowadays there is a lot of focus put on the use of natural compounds having anti oxidative and anti-apoptotic properties to amplify the effectiveness in reducing the toxicity induced by chemotherapeutic drugs (Khan et al., 2012a,b). It has been

Abbreviations: 5-FU, 5-Flourouracil; CH, chrysin; KIM-1, kidney injury molecule; BSA, bovine serum albumin; CDNB, 1-chloro 2, 4-dinitrobenzene; DTNB, 5,5′-dithio bis-[2-nitrobenzoic acid]; EDTA, ethylene diamine tetra acetic acid; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; NADPH, reduced nicotinamide adenine dinucleotide phosphate; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; MDA, malondialdehyde.

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reported from various chemopreventive and epidemiological studies in animal models and humans that the consumption of fruits and vegetables in diet decrease risk of cancer due to the presence of various essential nutrients and phenolics predominantly flavoniods (Surh et al., 2001 and Middleton et al., 2000).

Flavonoids are natural phenolic compounds of plant origin representing an extensive array of biological characteristics including antibacterial, anti-inflammatory, anti allergic, antithrombotic and vasodilatory properties (Bosetti et al., 2007). At present much attention is being drawn towards alternative medicine because of the use of dietary supplements and herbal preparations for maintenance of health and prevention of disease. Henceforth there is an increased interest in deciphering the biological roles of flavonoids, which are found to be major constituents of some traditional medicinal plants (Wang and Morris, 2007).

CH (5, 7-dihydroxyflavone) is a natural flavonoid which possesses wide variety of biological activities and is found in many plant extracts including propolis, blue passion flower and honey. Recently, a number of studies have shown that CH is antioxidant, anti inflammatory and anti cancerous in nature. It has been reported *in vitro* to induce apoptosis in a panel of cancer cell lines, including HeLa cervical cancer cells, U937, HL-60 and L1210 leukemia cells (Li et al., 2010). It also down regulates the expression of proliferating cell nuclear antigen (PCNA) by inducing apoptosis through caspase activation, Akt inactivation in U937 leukemia cells and causes cell-cycle arrest in human colon cancer cells, C6 glioma cells (Miyamoto et al., 2006). No information is available on chemopreventive potential of CH against 5-FU induced renal toxicity. In this study, we found that CH administration significantly down regulated the level of pro-apoptotic factors like p53, Bax, caspase-3 and up regulated Bcl-2 simultaneously reducing chemotherapy induced apoptosis. This provides a new evidence to support a novel approach towards cancer therapeutics.

2. Materials and methods

2.1. Chemicals

GR, GSSG, GSH, DTNB, CDNB, BSA, NADP, NADPH, FAD, TBA, TCA, FCR, 5-FU, etc. were obtained from Sigma–Aldrich, USA. All other chemicals and reagents were of the highest purity grade and commercially available.

2.2. 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 ad libitum. 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).

2.3. Treatment protocol

Rats were randomly divided into five groups of six rats each. Group I served as control and received corn oil for 21 days (10 ml/kg) and 0.9% saline intraperitoneally on 19th. Group II served as positive control and received intraperitoneal injections of 5-FU (150 mg/kg b.wt.) on the 19th day. Groups III and IV were co-treated with an oral dose of 5-FU 50 mg/kg b.wt. (D1) and 100 mg/kg b.wt. (D2), respectively, for 21 days and intraperitoneal injection of 5-FU (150 mg/kg b.wt.) was administered on 19th day in both the groups. Group V received only D2 (100 mg/kg b.wt.) of 5-FU for 21 days. On the 21st day, the rats were sacrificed and kidney samples were taken after perfusion for examination of various biochemical, immunohistochemical and histo pathological parameters. Before sacrifice, rats were given mild ether anesthesia. Later on, blood was drawn 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.

In vivo protocol

Groups	Treatment from 1st to 21st day	Treatment on 19th day
Group I (control)	Corn oil (10 ml/kg)	Normal saline only (0.9%)
Group II (only 5-FU)	Corn oil (10 ml/kg)	5-FU 150 mg/kg b.wt.i.p (19th day)
Group III (5-FU + CHD1)	CH 50 mg/kg b.wt.	5-FU 150 mg/kg b.wt.i.p (19th day)
Group IV (5-FU + CHD2)	CH 100 mg/kg b.wt.	5-FU 150 mg/kg b.wt.i.p (19th day)
Group V (only CHD2)	CH 100 mg/kg b.wt.	CH 100 mg/kg b.wt.

2.4. Tissue processing

Post mitochondrial supernatant of kidney samples was prepared by the method of Khan and Sultana, 2011. In brief, the kidneys were removed quickly, cleaned of extraneous material and immediately perfused with ice-cold saline (0.85% NaCl). The kidneys were homogenised in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%) using a Potter–Elvehjen homogeniser. The homogenate was filtered through muslin cloth and centrifuged at 800*g* for 5 min at 48C by a REMI cooling centrifuge to separate the nuclear debris. The aliquot obtained was centrifuged at 12,000 rpm for 20 min at 4 °C to obtain the PMS, which was used as a source of enzymes.

2.5. Assay for CAT activity

The CAT activity was assessed by the method of Claiborne (1985). In short, the reaction mixture consisted of 0.05 ml PMS, 1.0 ml of H_2O_2 (0.019 M), 1.95 ml 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 H_2O_2 consumed/min per mg protein.

2.6. Assay for lipid peroxidation

The assay of lipid peroxidation (LPO) was done according to the method of Wright et al. The reaction mixture consisted of 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml microsome, 0.2 ml ascorbic acid (100 mM) and 0.02 ml ferric chloride (100 mM), in a total volume 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 trichloroacetic acid (10%). Following the addition of 1.0 ml thiobarbituric acid (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 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 nmol TBA formed/h per g tissue at 378C by using a molar extinction coefficient of $1.56 * 105$/M per cm.

2.7. Assay for SOD activity

The SOD activity was measured by the method of Marklund and Marklund (1974). The reaction mixture consisted of 2.875 ml Tris–HCl buffer (50 mM, pH 8.5), pyrogallol (24 mM in 10 mM-HCl) and 100 ml PMS, in a total volume of 3 ml. Enzyme activity was measured at 420 nm and was expressed as units/mg protein. One unit of enzyme is defined as the enzyme activity that inhibits the autooxidation of pyrogallol by 50%.

2.8. Assay for GSH

GSH was assessed by the method of Jollow et al. A quantity of 1.0 ml of 10% PMS mixed with 1.0 ml of (4%) sulphosalicylic acid was taken, incubated at 4 °C for a minimum time period of 1 h and then centrifuged at 4 °C at 1200 g for 15 min. The reaction mixture of 3.0 ml was composed of 0.4 ml of supernatant, 2.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.4 ml dithio-bis-2-nitrobenzoic acid (4 mg/ml). The yellow colour developed was read immediately at 412 nm on the spectrophotometer (Lambda EZ201; Perkin Elmer). GSH concentration was calculated as nmol GSH conjugates/g tissue.

2.9. Assay for GPx activity

The activity of GPx was calculated by the method of Mohandas et al. (1984). The total volume of 2 ml was composed of 0.1 ml EDTA (1 mM), 0.1 ml sodium azide (1 mM), 1.44 ml phosphate buffer (0.1 M, pH 7.4), 0.05 ml glutathione reductase (1 IU/ml is equivalent to 1 mol GSSG reduced/min per ml), 0.05 ml GSH (1 mM), 0.1 ml NADPH (0.2 mM), 0.01 ml H_2O_2 (0.25 mM) and 0.1 ml of 10% PMS. The depletion of NADPH at 340 nm was recorded at 25 °C. Enzyme activity was calculated as nmol NADPH oxidised/ min per mg protein with a molar extinction coefficient of $6.22 * 10^3$/M per cm.