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Original Article

Abrogation of 5-flourouracil induced renal toxicity by bee propolis via targeting oxidative stress and inflammation in Wistar rats

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ABSTRACT

Background: 5-fluorouracil (5-FU) is a strong anticancer agent however its clinical use is constrained because of its marked organ toxicity associated with increased oxidative stress and inflammation. The present study was designed to investigate the protective effects of bee propolis (BP), a naturally occurring bioflavonoid against 5-FU induced renal toxicity in Wistar rats using biochemical, histopathological changes and expression levels of inflammation.

Methodology: Wistar rats were subjected to concomitant pre and post-phylactic oral treatment of BP (80 and 160 mg/kg b.wt.) against nephrotoxicity induced by i.p. injection of 5-FU (75 mg/kg b.wt) and were sacrificed after 48 h. Nephrotoxicity was assessed by measuring the level of serum creatinine, BUN, LDH, KIM-1 and TNFα. The level of anti-oxidant defense enzymes of kidney tissue was also measured.

Results: Treatment with BP decreased the levels of serum toxicity markers significantly and additionally induced anti-oxidant defense enzyme levels as well as decreased expression of NFkB. Histopathological changes further confirmed the biochemical and immunohistochemical results showing that 5-FU caused significant structural damage to kidneys like tubular necrosis, renal lesions, glomerular congestion and dilated blood sinusoids were also observed. All these features of 5-FU induced toxicity were reversed by co-administration of BP.

Conclusion: Therefore, our study favors the view that BP may be a useful modulator in alleviating 5-FU induced nephrotoxicity.

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1. Introduction

5-FU is an antineoplastic agent, belongs to the group called antimetabolites and functions as a pyrimidine analog, synthesized by Heidelberg some 50 years ago.[1] It has been used extensively in the treatment of patients with breast, stomach, colorectum, head and neck, genitourinary tracts, glaucoma and skin cancer.[2] Although it generates adequate effect, it further exhibits severe toxicity and detrimental side effects like leukopenia, diarrhea, stomatitis, alopecia, mucositis,[3] cardiotoxicity,[4] nephrotoxicty and hepatotoxicity.[5] It results in DNA damage, proliferative inhibition and apoptosis both in rapidly dividing cells including cancer cells and some normal dividing cells.[6] In this context, they often induce side effects in cancer patients that severely limit their activity.[7] Concisely, chemotherapy commences with the generation of oxidative stress and reactive oxygen species (ROS) which act to directly damage cells and tissues. Secondly, the transcription factor, nuclear factor kappa B (NFκB) is activated and leads to upregulation of many genes, including those responsible for the production of proinflammatory cytokines[8] like TNFα. Thus, chemicals with anti-inflammatory/antioxidative properties and minimal side effects may serve as potential therapeutic agents for the treatment. Free radical generation during treatment with 5-FU, leading to lipid peroxidation and cell membrane damage, could be one mechanism behind the toxic effects of 5-FU.[4]

BP is a well known ancient folk medicine, an intricate resinous hive product, and a blend of waxes, sugars and plant exudates collected by bees from plants. Flavonoids, aromatic acids, diterpenic acids and phenolic compounds appear to be the principal components responsible for its biological activities. It is alleged to exhibit a broad spectrum of activities including antibacterial, antifungal, antiviral, anti-inflammatory, local-anesthetic, anti-oxidant, immune stimulating, cytostatic and free radical scavenging activities.[9] Recently, it is also being used in food and beverages to improve health and prevent diseases such as inflammation, heart disease, diabetes and cancer.[10]

To the best of our knowledge such an extensive study on renal toxicity by 5-FU has been reported for the first time.

2. Materials and methods

2.1. Chemicals

Glutathione reductase, oxidized (GSSG) and reduced glutathione, 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), flavine adenine dinucleotide, 2,6-dichlorophenolindophenol, thiobarbituric acid (TBA), 5-FU etc: were obtained from Sigma–Aldrich, USA. Sodium hydroxide, ferric nitrate, trichloroacetic acid (TCA) and perchloric acid (PCA) etc were purchased from CDH, India. Plant extract was purchased from Saiba Industries, Mumbai.

2.2. Animals

Male Wistar rats (150–200 g), 6–8 weeks old, were obtained from the Central Animal House Facility of Hamdard University. 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 water for 28 days and 0.9% saline intraperitoneally (i.p.) on day 25th, 26th. Group II received i.p. injections of 5-FU (75 mg/kg b.wt.) on 25th and 26th day. Groups III and IV were treated with an oral dose of BP 80 mg/kg b.wt. (D1) and 160 mg/kg b.wt. (D2), respectively, for 28 days and i.p. injections of 5-FU (75 mg/kg b.wt.) were administered on 25th and 26th day. Group V received only D2 (160 mg/kg b.wt.) of BP for 28 days. On the 28th day, the rats were sacrificed by cervical dislocation, blood was drawn for serum parameters and kidneys were taken after perfusion for examination of various biochemical, immunohistochemical and histopathological parameters.

2.4. Biochemical estimation

Assay for catalase (CAT), lipid peroxidation (LPO), Superoxide dismustase (SOD), reduced glutathione (GR), glutathione peroxidase (GPx), glutathione reductase (GR), Blood Urea Nitrogen (BUN), Creatinine, lactate dehydrogenase (LDH) and protein estimation was done by the method as described by Rehman et al.[11]

2.5. Assay for tumor necrosis factor (TNFα) and KIM-1(Kidney injury molecule-1)

The level of TNF-α was quantitated using an ELISA based kit (eBioscience, Inc., San Diego., USA) and KIM-1 (RAT KIM-1 ELISA KIT, Adipo Bioscience, Inc, USA) following instructions of the manufacturer.

2.6. Immunohistochemistry

Kidney sections on polylysine coated slides obtained were fixed in neutral buffered formalin, and embedded in paraffin and were treated for NFkB antibody for immunohistochemical analysis. The procedure was processed according to the manufacturer's protocol recommended for NFkB immunohistochemistry with slight modifications.

2.7. Histopathological examination

The kidneys were quickly removed after sacrifice and preserved in 10% neutral buffered formalin for histopathological processing. The kidneys were embedded in paraffin wax and longitudinally sectioned with a microtome. Hematoxylin and