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Chemopreventive efficacy of hesperidin against chemically induced nephrotoxicity and renal carcinogenesis via amelioration of oxidative stress and modulation of multiple molecular pathways

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ABSTRACT

In the present study, chemopreventive efficacy of hesperidin was evaluated against ferric nitrilotriacetate (Fe-NTA) induced renal oxidative stress and carcinogenesis in wistar rats. Nephrotoxicity was induced by single intraperitoneal injection of Fe-NTA (9 mg Fe/kg b.wt). Renal cancer was initiated by the administration of N-nitrosodiethylamine (DEN 200 mg/kg b.wt ip) and promoted by Fe-NTA (9 mg Fe/kg b.wt ip) twice weekly for 16 weeks. Efficacy of hesperidin against Fe-NTA-induced nephrotoxicity was assessed in terms of biochemical estimation of antioxidant enzyme activities viz. reduced renal GSH, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, catalase, superoxide dismutase and renal toxicity markers (BUN, Creatinine, KIM-1). Administration of Fe-NTA significantly depleted antioxidant renal armory, enhanced renal lipid peroxidation as well as the levels of BUN, creatinine and KIM-1. However, simultaneous pretreatment of hesperidin restored their levels in a dose dependent manner. Expression of apoptotic markers caspase-3, caspase-9, bax, bcl-2 and proliferative marker PCNA along with inflammatory markers (NFκB, iNOS, TNF-α) were also analysed to assess the chemopreventive potential of hesperidin in two-stage renal carcinogenesis model. Hesperidin was found to induce caspase-3, caspase-9, bax expression and downregulate bcl-2, NFκB, iNOS, TNF-α, PCNA expression. Histopathological findings further revealed hesperidin's chemopreventive efficacy by restoring the renal morphology. Our results provide a powerful evidence suggesting hesperidin to be a potent chemopreventive agent against renal carcinogenesis possibly by virtue of its antioxidant properties and by modulation of multiple molecular pathways.

1. Introduction

Kidney cancer accounts for approximately 2% of all cancers and about 273,000 new cases of renal cancer are detected every year worldwide (Ferlay et al., 2010). Renal cell carcinoma (RCC) is one of the most common type of solid tumor representing about 80–85% of all renal malignancies (Jemal et al., 2006). RCC is associated with numerous risk factors and may be the result of exposure to different environmental toxicants (Jemal et al., 2010). Nitrilotriacetic acid (NTA), a synthetic tricarboxylic acid is one such toxicant used mainly as a substitute for polyphosphates in detergents and is a common water contaminant (Nancharaiah et al., 2006). Iron is the most essential nutritional element required for many biological processes and its deficiency may result in many diseases. However, increased iron overload has been known to be directly correlated with carcinogenic events (Smith et al., 1990). The possible mechanism underlying iron mediated carcinogenesis is the induction of oxidative stress resulting in free radical generating reactions, modulation in the immune system mechanism and potentiation of tumor growth (Inoue and Kawanishi, 1987). Iron may form complexes with nitrilotriacetic acid to form Fe-NTA. The iron-chelate of nitrilotriacetate, ferric nitrilotriacetate (Fe-NTA) is a strong nephrotoxic agent that induces renal proximal tubular necrosis (Ahmad et al., 2011). Epidemiological studies have suggested that renal toxicity associated with Fe-NTA is the result of increase in serum free iron levels and its subsequent reduction on the luminal side of the proximal tubule leading to the formation of reactive oxygen species causing lipid peroxidation and DNA damage (Umemura et al., 1990). It has been demonstrated that repeated Fe-NTA administration causes renal adenocarcinoma in rats (Iqbal et al., 2003).

Several treatment modalities of renal cell carcinoma include surgery and radiation but these are effective only against local neoplasms. RCC is

Abbreviations: RCC, Renal cell carcinoma; DEN, Diethylnitrosamine; Fe-NTA, Ferric nitilotriacetate; GPx, glutathione peroxidase; GSH, glutathione; GR, glutathione reductase; GST, glutathione-S-transferase; LPO, lipid peroxidation; MDA, malondialdehyde; PMS, post-mitochondrial supernatant; SOD, superoxide dismutase; BUN, blood urea nitrogen; KIM-1, kidney injury molecule; HS, hesperidin.

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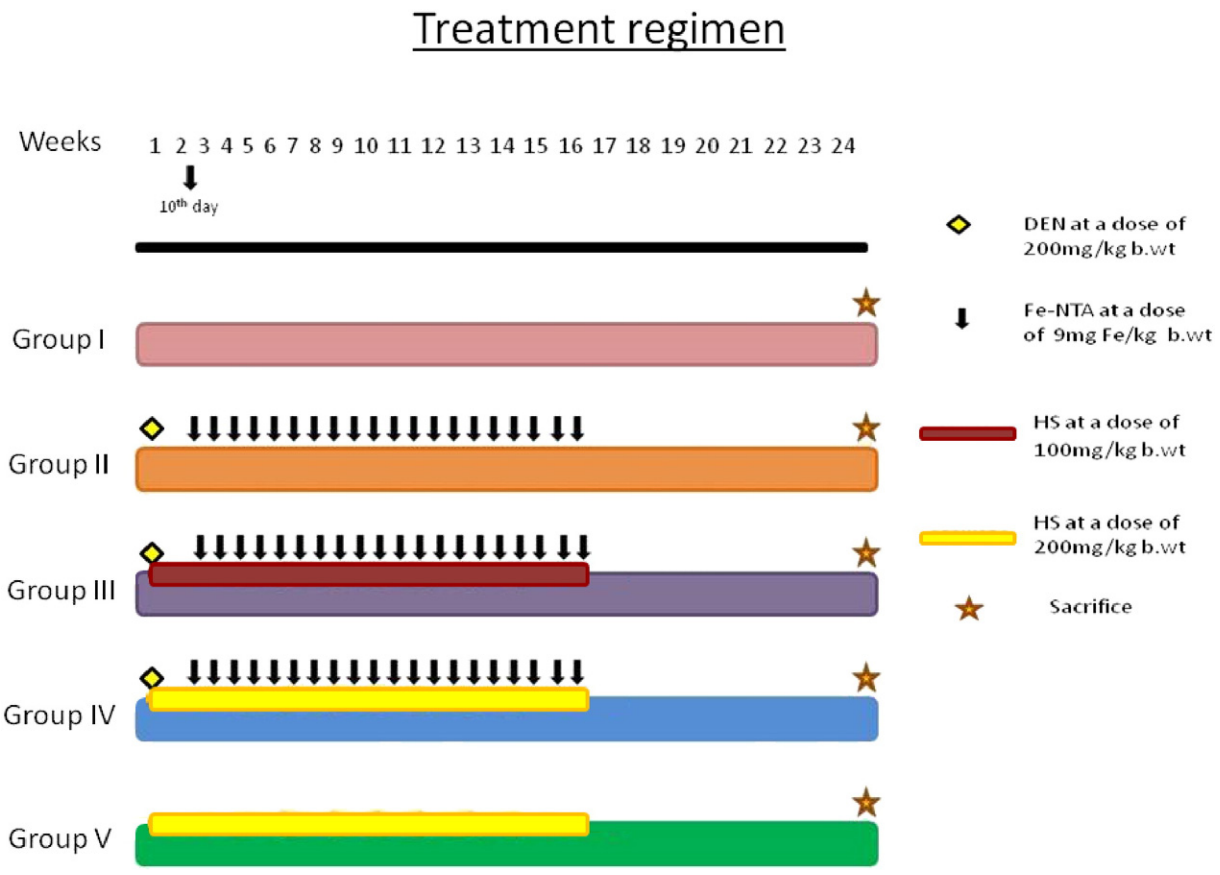

Fig. 1. Treatment regimen for tumor study. Animals were divided into five groups of 25 rats in each. Group I served as untreated control was given distilled water once daily, for 16 weeks. Rats in group II were initiated with DEN (200 mg/kg b.wt IP) on 1st first day and from 10th day onwards received Fe-NTA (9 mg Fe/kg b wt IP) twice a week for 16 weeks. Rats in group III received the same treatment as in group II but also received HS at a dose of 100 mg/kg b.wt orally once daily for a period of 16 weeks. Rats in group IV received the same treatment as in group II but also received HS at a dose of 200 mg/kg b.wt orally once daily for a period of 16 weeks. Group V was administered HS at a dose of 200 mg/kg b.wt orally once daily for 16 weeks. All the animals were sacrificed at the end of 24 weeks and processed for different assays.

relatively resistant to radiation therapy and responds very poorly to hormonal therapy and chemotherapy (Walsh et al., 2003). The response rate to immunotherapeutic agents such as interferon alpha (INF-α) and interleukin (IL-2) is also not very effective. Hence, the need to find an alternative strategy to suppress and control the development of carcinogenesis is very important. In this regard, plant products and natural compounds have shown a promising role in cancer chemoprevention.

Chemoprevention is the long-term intervention with natural or synthetic molecules to prevent, inhibit or reverse carcinogenesis and has proved to be an effective strategy in cancer prevention (Janani et al., 2010). Phytochemicals are known for their wide range of medicinal uses and are generally considered safe without any toxic side-effects. Many studies have suggested an inverse correlation between the high consumption of fruits and vegetables and cancer risk (Knekt et al., 2002; Manson, 2003).

Flavonoids are polyphenolic compounds known to possess anti-inflammatory, antitumor and antioxidant activities (Izzi et al., 2012; Kay et al., 2012). They are known to prevent many diseases (Clere et al., 2011) Hesperidin (HS) is a flavone glycoside present in citrus fruits. It is known to possess great medicinal value and have pharmacologically been deciphered to be an antioxidant, anti-inflammatory, antimicrobial, analgesic and immunomodulatory (Garg et al., 2001). Hesperidin has been reported to prevent oxidative stress in rat kidney (Wilmsen et al., 2005; Saiprasad et al., 2013; Tirkey et al., 2005). It has also been demonstrated to be hepatoprotective and nephroprotective against various toxicants (Kaur et al., 2006; Anandan and Subramanian, 2012). A number of studies have suggested that hesperidin possess chemopreventive potential against prostate cancer, lung cancer, skin cancer and colon cancer (Lee et al., 2010; Kamaraj et al., 2011; Saiprasad et al., 2013). A recent study conducted in humans also

Table 1
Effect of the hesperidin (HS) treatment on the antioxidant enzymes glutathione (GSH), glutathione S-transferase (GST), and glutathione peroxidase (GPx) and glutathione reductase (GR) on Fe-NTA-induced renal redox imbalance.

Treatment groups	GSH (nmol GSH/g tissue)	GST (nmol CDNB conjugate formed/ min/mg protein)	GPX (nmol NADPH oxidized/ min/mg protein)	GR (nmol NADPH oxidised/ min per mg protein)
Group I	0.625 ± 0.02	342.7 ± 18.95	141.48 ± 6.9	360.74 ± 9.42
Group II	0.319 ± 0.05$^{###}$	175.03 ± 9.3$^{###}$	58.59 ± 7.5$^{###}$	202.17 ± 3.35$^{###}$
Group III	0.54 ± 0.06**	259.10 ± 16.6*	120.54 ± 3.2***	241.77 ± 10.31***
Group IV	0.59 ± 0.02***	331.79 ± 10.7***	132.51 ± 4.9***	338.79 ± 2.32***
Group V	0.63 ± 0.01	343.65 ± 13.6	142.37 ± 10.09	362.81 ± 19.38

Results represent mean ± SEM of six animals per group. Group I — control (Distilled water only); Group II (toxicant) — TCE (1000 mg/kg); Group III — TCE (1000 mg/kg) + HS (100 mg/kg b.wt); Group IV — TCE (1000 mg/kg) + HS (200 mg/kg b.wt); Group V — HS (200 mg/kg b.wt). Results obtained are significantly different from control group ($^{###}p < 0.001$). Results obtained are significantly different from Fe-NTA treated group ($^{*}p < 0.05$, $^{**}p < 0.01$) and ($^{***}p < 0.001$).