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Original article

Umbelliferon-α-D-glucopyranosyl-($2^{I} \rightarrow 1^{II}$)-α-Dglucopyranoside ameliorates Diethylnitrosamine induced precancerous lesion development in liver via regulation of inflammation, hyperproliferation and antioxidant at pre-clinical stage

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ABSTRACT

It is well documented that anomalous production of inflammatory proteins linked with most of the toxic expression and genesis of diverse chronic disease including cancer. Diethylnitrosamine (DEN) a well-known hepatotoxin and hepatocarcinogen, can induce oxidative stress and inflammatory reaction in it. Umbelliferone, secondary metabolites, is present in different plants and widely consumed by humans as medicine and food supplements. The aim of the current study was to scrutinize the chemoprotective potential of umbelliferon-α-D-glucopyranosyl-($2^{I} \rightarrow 1^{II}$)-α-D-glucopyranoside (UFD) against DEN-induced hepatocellular carcinoma (HCC) in experimental rats. Single intraperitoneal injection of DEN (200 mg/kg) was used for induction of HCC in rats and rats were grouped and orally treated with UFD (5, 10 and 20 mg/kg) dose for 22 weeks. Parameters under investigation included hepatic, non-hepatic enzymes, oxidative stress, pro-inflammatory cytokines, COX-2 and NF-κB level along with histopathological examination in HCC rats. UFD exerted protective effect via reduction of oxidative stress, liver and non-liver parameters in a dose-dependent manner. It also reduced the expression of TNF-α, IL-1β, IL-6 and COX-2 in diseased rats. Our result revealed the essential repression of the inflammation cascade through modulation of nuclear factor-kappa B (NF-κB) signaling pathway.

1. Introduction

Hepatocellular carcinoma (HCC) is the 5th most common cancer along with other types of cancers prevailing worldwide. It accounts for about 90% of all solid tumors with more than 4% of all kinds of cancer cases that results in 4,40,000 new cases every year. The effective possible treatment available for HCC is liver transplantation and surgical resection but limited to few patients with early detection of HCC. Other alternative treatments like radiotherapy, surgery, hormone, immune and chemotherapy are also available with hospitals but with no great results in long run. The choice of cancer treatment mainly depends on type and stage of cancer. The treatment of HCC is particularly challenging because

Abbreviations: DEN, diethylnitrosamine; HCC, hepatocellular carcinoma; UFD, umbelliferon-α-D-glucopyranosyl-($2^{I} \rightarrow 1^{II}$)-α-D-glucopyranoside; NF-κB, nuclear factor-kappa B; CYP2E1, cytochrome P450; ROS, reactive oxygen species; DNA, deoxyribonucleic acid; COX-2, cyclooxygenease-2; PGE_2, prostaglandin; CPCSEA, committee for the purpose of control and supervision of experiments on animals; AFP, alpha-fetoprotein; AST, aspartate aminotransferase; ALT, alanine transaminase; ALP, alkaline phosphates; NO, nitric acid; WBC, white blood cells; RBC, red blood cells; Hb, hemoglobin; ESR, erythrocytes sedimentation rate; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; CAT, catalase; GPx, glutathione peroxidase; LPO, lipid peroxidase; SOD, superoxide dismutase; GST, glutathione-S-transferase; Glc6PD, glucose 6-phosphate dehydrogensae; CD, conjugated dienes; TNF-α, tumor necrosis factor-α; IL-6, Interlukin-6; IL-1β, Interlukin-1β; SEM, standard error mean; NC, normal control; H_2O_2, hydrogen peroxide; ALAD, aminolevulinic acid dehydratase.

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lot of factors which affect the HCC treatment are to be taken care of such as patient medical history, hepatic tissue and the various factor of the tumor. The said treatments have high toxic rate and can develop the drug resistance, both in tumor and normal cells. Further major side effects viz., thrombocytopenia, diarrhea, constipation, nausea, and alopecia etc are also associated with available chemotherapy. Chemoprevention is a major approach for the management of cancer by targeting the various receptors, which are involved in cancer progression and initiation [1]. Cancer chemoprevention also finds the pharmacological intervention of natural origin compounds and synthetic products, which may inhibit the abnormal growth, prevent carcinogenesis and inhibit the development of invasive cancer [2]. Many epidemiology studies suggest that dietary consumable products (fruit, vegetable, and herbal medicine) have potential to inhibit the growth of cancer in cost efficient manner.

For assessing, the efficacy of drugs on HCC, generally tumor inoculation or chemicals is used for induction HCC. Usually, DEN and aflatoxinB1, are used to induce the hepatocarcinoma [3]. These chemicals are found in agriculture, tobacco, pharmaceutical products along with cosmetic preparation exposed to humans [4,5]. Aflatoxin B1 takes long to (45 weeks) that can develop liver cancer with 35–50% induction rate. DEN takes few weeks to develop the tumor with 100% induction rate. DEN is a liver-specific carcinogen from the family of N-nitroso compounds, known to induce mutagenesis, teratogenesis, and carcinogenesis by DNA repair/replication; commonly used to produce the tumor in all animal species. DEN induces the hepatic necrosis via activation of cytochrome P450 (CYP2E1) in experimental rats. After the metabolic biotransformation nitrosamine induces hepatic carcinogenesis by O4- and O6-ethyl deoxythymidine and O6-ethyl deoxyguanosine. CYP2E1 stimulate the Kupffer cells, which leads the production of reactive oxygen species (ROS) (hydroxyl, superoxide, peroxyl as well as non-radicals, including peroxynitrite and hydrogen peroxide) that damage the liver cells by nitrosamine [6,7]. The elevated level of ROS results in either injury to the cell or cellular component or activation of signal transduction pathway, which produces hepatic cells damage [8]. ROS generate the oxidative stress induce cell death via lipid peroxidation, DNA fragmentation and carbonylation [9]. Several evidence shows that excess generation of oxidative stress encourages the angiogenesis by alterations in redox signaling, which play a critical role in cellular proliferation, signal transduction, apoptosis and differentiation [10]. Another mechanism for nitrosamine-induced hepatocarcinoma is increased level of free radicals in the cell, these free radicals burst release and lead to cellular injury and oxidative stress. Further increase the level of free radical initiates the pathological alteration, including tumor expansion, carcinogenicity, hepatocellular necrosis and neoplastic changes [11].

A lot of factors such as chemical carcinogen, obesity, and chronic virus infection has deliberated promotion to Hepatocellular Carcinoma (HCC). HCC is the inflammation related tumor that is endorsed to above-mentioned risk factors. Furthermore, some reports are available that correlates the overexpression of cyclooxygenease-2 (COX-2) and various types of cancers [12,13]. COX-2 is the rate-limiting enzyme, that causes the mitogenic stimuli and inflammation; which results in increased production of prostaglandin synthesis in neoplastic tissue and inflamed area [14]. Hepatitis C and B virus, Aflatoxin B1 and diethylnitrosamine (DEN) [12,15,16] induced the COX-2 expression in HCC, which facilitate the cancer expansion and development of hepatocarcinoma [12,15]. HCC also involves enzymes assisting in inflammatory process mainly the pro-inflammatory cytokines; they serve as the rate-limiting enzyme in prostaglandin (PGE_2) biosynthesis from arachidonic acid. Several experimental studies showed that the increase expressions of pro-inflammatory cytokines in carcinogenesis are due to nuclear factor kappa B (NF-κB). Modulation of abnormal up-regulation of NF-κB has been identified as the molecular basis of chemoprevention by the use of dietary phytochemicals, in humans. Therefore, inhibition of pro-inflammatory cytokines and modulation of abnormal up-regulation of NF-κB has now been recognized as a target for the molecular basis of chemoprevention by structurally diverse dietary phytochemicals. Hence, suppression of pro-inflammatory cytokines can be considered as a new paradigm in cancer chemoprotective in several organs.

Various experimental, epidemiological and clinical studies confirmed that the free radical scavenger rich diet can reduce the circumstance of chronic diseases particularly cancer [1,12,14,16]. Umbelliferone (7-hydroxycoumarin) is considered as the potent free radical scavenger, due to its free radical scavenging activity is already proved by us for its various pharmacological action such as antioxidant, anti-hyperlipidemic, anti-nociceptive and anti-diabetic effect in rats [17,26]. It also showed the protective effect against inflammation, arthritis, renal cancer and inhibitory effect against laryngeal cancer cells. Kumar et al., isolate the UFD from the *Aegle marmelos* plant and already proved the antioxidant and anti-inflammatory potential [17]. Previous research suggested that the UFD is a potent antioxidant and anti-inflammatory molecules. Due to the anti-inflammatory and antioxidant nature of UFD, the authors made the efforts to decipher the effect of UFD against DEN-induced hepatocarcinogenesis by suppressing the NF-κB pathways.

2. Material

2.1. Chemicals

Diethylnitrosamine (DEN) was purchased from the Sigma-Aldrich Chemical Company, USA. COX-1 and COX-2 ELISA kits were purchased from the Cayman Chemicals Ltd USA. All chemicals, reagents, and kits were of analytical grade and purchased from the approved vendors.

2.2. Isolated compound

In the continuation of our research, in the current study, we used our previous isolated compound Umbelliferon-α-D-glucopyranosyl-($2^{I} \rightarrow 1^{II}$)-α-Dglucopyranoside (UFD) from the stem bark of Aegle marmelos Corr [17].

2.3. Experimental animals

Pathogen-free adult male Wistar (Swiss albino strain) rats, initially weighing 100–135 gm, were acquired from the Departmental animal house facility for experimental purpose. The rats were acclimatized, as per the procedure of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Ministry of Environment and Forests, Government of India. The experimental study was approved form the IAEC-SHIATS vide approval number (IAEC/SHIATS/PA15IX/FVK01).

2.4. Induction of hepatocellular carcinoma (HCC)

DEN (200 mg/kg) was prepared in phosphate buffer solution and intraperitoneally administered to animals. HCC was confirmed by the estimation the alteration in alpha-fetoprotein (AFP) level after the 14 days [20].