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Formylchromone exhibits salubrious effects against nitrosodiethylamine mediated early hepatocellular carcinogenesis in rats

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ABSTRACT

The salubrious effects of 3-Formylchromone (3-FC) against nitrosodiethylamine (NDEA) mediated early hepatocellular carcinogenesis was investigated *in vivo* by this study. Male Wistar rats were administered three interspersed intraperitoneal injections of NDEA (200 mg/kg body weight) until sixth week, followed by, thrice a week oral dose of 3-FC (25 mg/kg body weight) from the seventh week to eleventh week. Oral supplementation of Wistar rats with 3-FC prevented the increase in serum marker enzymes (AST, ALT, LDH) and serum pre-neoplastic marker (γ-GT) induced by NDEA. Biochemical observations were found to be further correlated with histological studies, indicating the potential of 3-FC to mediate suppression of hepatic damage/pre-neoplastic lesions. Argyrophilic nucleolar organizer region (AgNOR) staining was done in histology sections to confirm the anti-proliferative potential of 3-FC against NDEA-induced early hepatocellular carcinogenesis. RT-PCR and immunoblot analysis was done to find the modulations in the gene transcript/protein level expression of pre-neoplastic marker (GST-pi), proliferation marker (PCNA), apoptotic mediators (PPARγ, NFκB-p65 and p53). 3-FC was found to favorably modulate the expressions of GST-pi, PCNA, PPARγ, NFκB-p65, p53 clearly confirming the anti-proliferative and apoptotic potential of 3-FC. Further, the apoptotic effect of 3-FC against NDEA-induced early hepatocellular carcinogenesis was confirmed by caspase-3 activity assay and DNA fragmentation analysis. Based on these findings, it is concluded that 3-FC possesses hepatoprotective, anti-pre-neoplastic, anti-proliferative and apoptosis inducing capability against NDEA-induced early hepatocellular carcinogenesis.

1. Introduction

Hepatocellular carcinoma (HCC) is a lethal solid tumor originating from the epithelial cells of the liver. They are the major histological class among other types of liver cancer. It is further found to account for approximately more than 80% of primary liver cancer cases [1]. Globally, HCC is the fifth leading cause of cancer incidence in men [2]. Nevertheless, HCC is also considered as the primary cause of mortality among cirrhotic patients [2]. Hepatitis B virus (HBV)/hepatitis C virus (HCV) mediated chronic infections are the major risk factors found to be associated with 80% of HCC cases. The environmental risk factors responsible for HCC occurrence include alcohol abuse or dietary intake of food contaminants such as aflatoxin B1 [3].

For cancer risk assessment, the standard method for evaluation of carcinogenicity and chemical toxicity is the long-term carcinogenicity test in the rats (2 years) [4]. Such tests are time-killing, arduous and expensive. In rats, an alternative method to long-term carcinogenicity test has been proposed featuring an early evaluation of pre-neoplastic lesions, which are accepted as end-point markers for the assessment of carcinogenicity [5,6]. Results are obtained with experimentally validated *in vivo* short-term liver bio-assay model of carcinogens in a matter of weeks, rather than the long-term model of carcinogenesis for many months [7,8].

In our study, initiation of early hepatocellular carcinogenesis in rats was performed by nitrosodiethylamine (NDEA) administration, referred to as the short-term liver bio-assay model [8]. This short-term model involves the development of pre-neoplastic lesions in rat liver, when NDEA is administered as three interspersed intraperitoneal injections, thrice a week, until the sixth week only [8]. NDEA mediated carcinogenesis in rats is considered as an ideal animal model to investigate liver tumor formation because it proceeds in stages similar to that of human HCC. NDEA is said to be responsible for the formation of pre-neoplastic lesions, followed by neoplastic nodules occurrence and then development

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of HCC nodules [9,10]. Additionally, NDEA is known to be present in certain occupational settings such as solvent in the fiber industry, as a softener for co-polymers and as an additive for lubricants in condensers. It is also present in diet, tobacco products, cosmetics, pharmaceutical products. NDEA is also found to be endogenously formed in the human body. Henceforth, it is also considered as potential risk factor for the development of HCC [11]. In spite of the numerous technological advancements and clinical facilities that are available for treatment of HCC, the cure for HCC still remains elusive. The limited therapeutic options for HCC and also its ensuing increase of incidence, morbidity, mortality are believed to warrant further studies to identify novel chemopreventive agents that can selectively influence a plethora of subcellular events [12]. Chromones are one such group of benzopyran derivatives that have long attracted numerous researchers from the point of view of both pharmacological activity and organic synthesis. Chromones (1-benzopyran-4-ones) and their derivatives are naturally occurring compounds ubiquitously found in the plant kingdom, and therefore are trusted to be present in representative amounts in a normal human diet [13]. Chromone derivatives are said to possess the chemical skeleton of naturally occurring flavonoids (1-benzopyran-4-ones) which have been extensively studied and have been found to possess myriad *in vitro* and *in vivo* pharmacological activities [13]. For our study, we have chosen one such chromone derivative 3-FC (Fig. 1) which has been very recently reported to exhibit anti-fungal, anti-viral, anti-microbial, anti-allergenic, anti-inflammatory and anticancer activity [14]. 3-FC is known to be implicated in the inhibition of NFκB, protein tyrosine phosphatase 1B, chelation of divalent cations, multidrug resistance proteins, p56 tyrosine kinase and thymidine phosphorylase [15–20]. It has also been shown *in vitro* to exhibit tumor cell specific cytotoxicity against leukemia, breast cancer and colon cancer based on the modulation of cell signaling pathways involved in proliferation and apoptosis [15,19]. Interestingly, it was known that activation of peroxisome proliferator-activated receptor gamma (PPARγ), a family of nuclear receptors, can exert anti-inflammatory effects in several cell types, such as hepatic cells, endothelial cells and macrophages [21,22]. Anti-proliferative and apoptotic properties of PPARγ include the potential to interfere with transcriptional pathways involved in inflammatory responses, such as modulation of NFκB signaling and thereby regulating tumor suppressor p53. Nevertheless, there were no randomized trial *in vivo* data available to address the modulating beneficial effect of 3-FC on supplementation and its ability to modulate the PPARγ–NFκB–p53 biological pathways during early hepatocellular carcinogenesis. Therefore, the current study was planned to investigate the chemo-preventive/salubrious role of 3-FC and decipher its molecular level potential to modulate anti-proliferative and apoptotic responses in the experimentally validated early hepatocellular carcinogenesis model.

2. Materials and methods

2.1. Chemicals

NDEA, 3-FC, ribonuclease A, proteinase K and ethidium bromide were purchased from Sigma (St. Louis, MO, USA). The antibodies for GST-pi, PCNA, PPARγ, NFκB-p65, p53 and GAPDH were purchased from Santacruz Biotechnology (USA). Chemiluminescence reagents

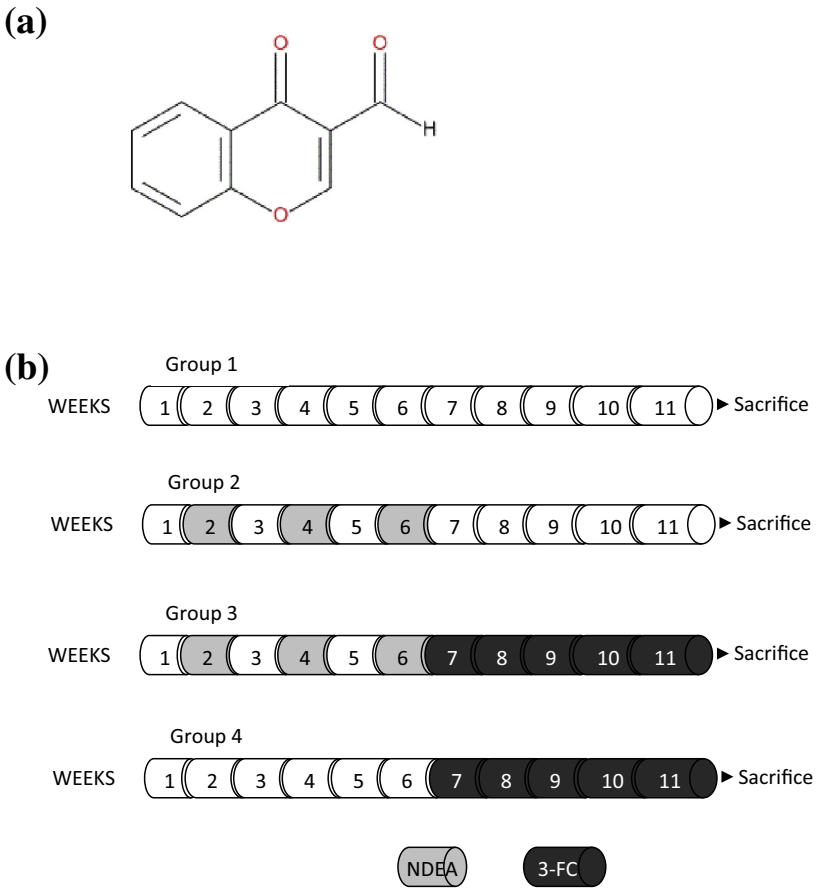

Fig. 1. (a) 3-Formylchromone (3-FC) structure. (b) Experimental design.