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Antioxidant and antitumor efficacy of Luteolin, a dietary flavone on benzo(a)pyrene-induced experimental lung carcinogenesis

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ABSTRACT

The present study is designed to assess the antioxidant and antitumor potential of luteolin against benzo (a)pyrene [B(a)P]-induced lung carcinogenesis in Swiss albino mice. Here, we reported that oral administration of B(a)P (50 mg/kg body weight) to mice resulted in raised lipid peroxides (LPO), lung specific tumor markers such as carcinoembryonic antigen (CEA) and neuron specific enolase (NSE) with concomitant decrease in the levels of both enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-s-transferase (GST), and non-enzymatic antioxidants such as reduced glutathione (GSH), vitamin E and vitamin C. Luteolin treatment (15 mg/kg body weight, p.o) significantly counteracted all these alterations and maintained cellular normalcy. Moreover, assessment of protein expression levels by western blot analysis revealed that luteolin treatment effectively negates B(a)P-induced upregulated expression of proliferating cell nuclear antigen (PCNA), cytochrome P450 1A1 (CYP1A1) and nuclear factor-kappa B (NF-κB). Furthermore, histopathology of lung tissue and immunohistochemistry of CYP1A1 were carried out to substantiate the anti- lung cancer effect of luteolin. Overall, these findings confirm the chemopreventive potential of luteolin against B(a)P induced lung carcinogenesis.

1. Introduction

Lung cancer is a major cause of morbidity and mortality worldwide in both men and women, accounting for 20% of all cancers [1]. Benzo(a)pyrene, a prototype lung carcinogen of poly aromatic hydrocarbon class, is known to induce enormous amounts of free radicals which results in oxidative stress that plays a crucial role in B(a)P-induced lung carcinogenesis [2]. The persisting grim of lung cancer mortality figures urge to discover novel approaches to control this deadly disease. In the recent years, dietary phytochemicals have fascinated the attention of researchers due to their promise of being powerful antioxidants that can protect human beings from free radical induced toxic effects. A large number epidemiological and experimental studies have convincingly proved that high dietary intake of fruits and vegetables rich in antioxidants have presented favourable effects in the chemoprevention of a multitude of diseases, including lung cancer [2,3].

Chemoprevention refers to the use of natural, dietary or synthetic compounds to prevent, reverse, or delay the development of cancer [4]. A number of effective chemopreventive measures have been introduced considerably to reduce both the incidence and mortality from lung cancer; among them use of dietary agents have gained immense interest for development as chemopreventive agents to treat lung cancer owing to their ubiquitous nature, inexpensiveness and broad safety window [2,4,5]. Luteolin is one such compound with promising role in cancer. Luteolin, 3′,4′,5,7-tetrahydroxyflavone (Fig. 1), a dietary phytochemical, belongs to a group of naturally occurring compounds called flavones, a type of flavonoids that are widely present

Abbreviations: B(a)P, benzo(a)pyrene; BPDE, B(a)P 7,8-diol-9,10-epoxide; CAT, catalase; CEA, carcinoembryonic antigen; CME, carboxy methyl cellulose; CYP1A1, cytochrome P450 1A1; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GST, glutathione-*S*-transferase; MDA, malondialdehyde; NF-κB, nuclear factor kappa-B; NSE, neuron-specific enolase; PCNA, proliferating cell nuclear antigen; ROS, reactive oxygen species; SOD, superoxide dismutase; TBST, tris buffer saline tween; Vit, vitamin.

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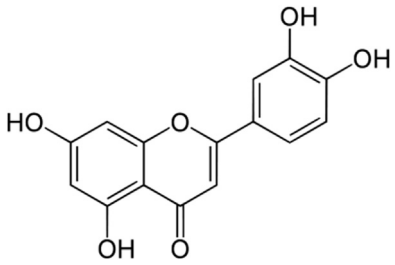

Fig. 1. Chemical structure of luteolin.

in fruits and vegetables such as apples, celery, peppers, parsley, onion leaves, broccoli, carrots and cabbages [6]. Luteolin, possess many beneficial properties including antioxidant, anti-inflammatory, cardio protective, anti-diabetic and anti-proliferative [6,7]. Luteolin has recently reported to show protective properties against several experimental carcinogenesis [8]. These studies have demonstrated that luteolin has promising potential as a chemopreventive agent and is worthy of further study. Therefore, the primary objective of the present study is to assess the antioxidant and antitumor efficacy of luteolin in B(a)P induced lung carcinogenesis in Swiss albino mice and to explore the possible mechanisms.

2. Materials and methods

2.1. Chemicals

Benzo(a)pyrene (purity, ~96%) and β-actin antibody were purchased from Sigma Aldrich (St Louis, MO, USA). Luteolin was purchased from TCI Company (Tokyo, Japan). The primary and secondary antibodies used in our experiment were procured from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All other chemicals and solvents used were of the highest analytical grade procured from commercial sources.

2.2. Animals

Healthy male Swiss albino mice, 6–8 weeks old, weighing 18–22 g were used in the study. Mice were procured from the central animal facility of the institute and housed in polypropylene cages. Animals were acclimatized for one week prior to the start of experiment and maintained on a standard housing condition under controlled atmosphere of 12 h light/dark cycles with an ambient temperature of 25 ± 2 °C and humidity at 50 ± 10%. Animals were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai under the trade name Amrut rat/mice feed) and water *ad libitum*. All the procedures with animals were strictly conducted according to the ethical norms approved by the Institutional Animal Ethical Committee (IAEC) regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.3. Experimental design

The study mice were randomly divided in to five groups of six animals in each as follows; (Fig. 2) Group I (Normal control)—animals received 0.5% carboxymethyl cellulose (CMC) orally throughout the course of experiment. Group II (Drug control)—animals received luteolin (15 mg/kg body weight dissolved in 0.5% CMC) orally thrice in a week for sixteen weeks to assess the cytotoxicity (if any) induced by luteolin. Group III (B(a)P control)—animals received B(a)P (50 mg/kg body weight dissolved in corn oil) orally twice a week for four successive weeks. Group IV (Luteolin pre-treatment)—animals received B(a)P (as in Group III) along with luteolin (15 mg/kg body weight dissolved in 0.5% CMC) orally thrice in a week for throughout the study period. Luteolin

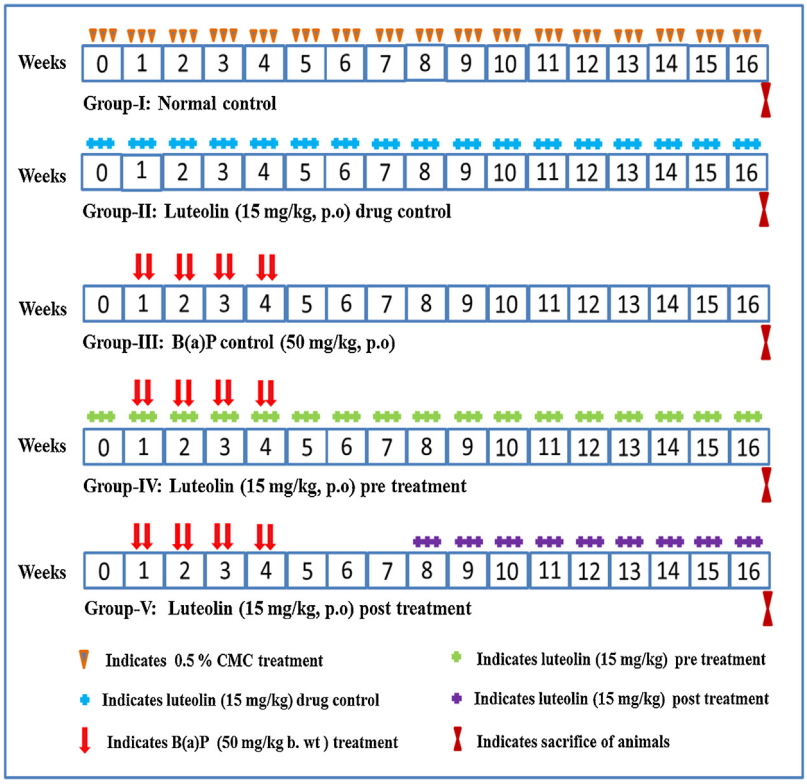

Fig. 2. Experimental design of the study.