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Protective effect of *p*-coumaric acid against 1,2 dimethylhydrazine induced colonic preneoplastic lesions in experimental rats

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ABSTRACT

Oxidative stress and gut microbial enzymes are intricately linked to the onset of colon carcinogenesis. Phytochemicals that modulate these two factors hold promise for the development of such agents as anticancer drugs. The present study evaluates the chemopreventive potential of *p*-coumaric acid (*p*-CA) – a phenolic acid in rats challenged with the colon specific procarcinogen DMH (1,2 di-methyl hydrazine). Rats were randomized into six groups (n = 7/group). Group 1 (control); Group 2 (*p*-CA 200 mg/kg b.w.); Group 3 (DMH 40 mg/kg b.w.); Groups 4 (DMH + *p*-CA 50 mg/kg b.w.) and Group 5 (DMH + *p*-CA 100 mg/kg b.w.) and Group 6 (DMH + *p*-CA 200 mg/kg b.w.). After the experimental duration of 15 weeks' rats were subjected to necropsy and tissues were collected for the histological and biochemical investigations. DMH induced colonic preneoplastic lesions viz., aberrant crypt foci (ACF), dysplastic ACF (DACF), mucin depleted foci (MDF) and beta catenin accumulated crypts (BCAC) were significantly suppressed by *p*-CA supplementation. Glucuronide conjugation of DMH in liver and its subsequent deconjugation mediated by microbes in the colon induced the formation of colonic preneoplastic lesions. *p*-CA inhibited these lesions and protected the rat colon against genotoxic insult by scavenging the free radicals via its strong antioxidant response and detoxification mechanism as measured by TBARS and enzymic antioxidants in control and experimental rats. Of the three tested doses, *p*-CA at a dose of 100 mg/kg body weight is found to exhibit a significant optimum effect compared to the other two doses 50 mg/kg body weight and 200 mg/kg body weight.

1. Introduction

Worldwide, colorectal cancer (CRC) remains the fourth most common cause of cancer related mortality with over 1.2 million new cases diagnosed each year along with >600,000 death per year [1]. In the United States in 2017, 135,430 individuals newly diagnosed with CRC and 50,260 deaths from the disease are projected [2]. According to the ICMR statistics, in India the annual incidence rates for colon cancer in men are 4.4 per 100,000 and in women are 3.9 per 100,000 [3]. This situation persists despite the fact that colon cancer is highly preventable by consumption of high fiber and low fat diet instead of high fat and low fiber diet. Chemoprevention involves the prevention or delaying the process of carcinogenesis through chronic administration of one or more chemical compounds and this chemoprevention concept is found to be the key in reducing the tumor burden and mortality [4]. Reactive oxygen species (ROS) plays a key role in microbiota-linked colon cancer via mechanisms that include bacterial-derived genotoxins, microbial-derived metabolism, the modulation of host defenses and inflammation pathways, oxidative stress induction, and anti-oxidative defense regulation [5]. Thus oxidative stress and gut microbiota are intricately linked to the onset of colon carcinogenesis. Phytochemicals that modulate these two factors holds promise for the development of such agents as anticancer drugs.

p-Coumaric acid (*p*-CA), a phenolic acid of the hydroxycinnamic acid family, is found ubiquitously in free or bound form in mushroom, fruits (e.g. apples, pears, grapes, oranges, tomatoes and berries), vegetables (e.g. beans, potatoes and onions) and cereals (e.g. maize, oats and wheat) and is shown to possess anti-oxidant, anti-inflammatory, anti-ulcer, antiplatelet, anti-cancer and anti-mutagenic properties [6,7]. *p*-CA was reported to inhibit cell

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proliferation by presumably affecting different cell cycle phases in the human colonic cell line Caco-2 [8] and by inducing apoptosis through ROS-mitochondrial pathway in colon cancer cells like HT 29 and HCT 15 [9].

This is the first study attempted to explore the short term exposure of *p*-CA in rats challenged with DMH at a dose of 40 mg/kg body weight for five weeks with a total experimental period of 15 weeks. This time period allows us to investigate the development of DMH induced preneoplastic lesions and the impact of *p*-CA on the same.

2. Materials and methods

2.1. Chemicals

1,2 dimethylhydrazine (DMH), *p*-Coumaric acid (*p*-CA), methylene blue and alcian blue were purchased from Sigma Chemical Co. (St Louis, MO, USA). β-catenin antibody and polymer HRP detection kit were purchased from BioGenex laboratories (San Ramon, CA, USA). All other chemicals and solvents used were of analytical grade and obtained from Hi-Media Laboratories (Mumbai, Maharashtra, India).

2.2. Animal care and diet

Male albino Wistar rats (140–150 g) were procured from the Central Animal Facility, SASTRA University, Thanjavur, India. Animals were acclimatized to the laboratory conditions for 1 week and maintained under standard conditions. The animals were given standard rat feed (Altromin, Germany) and water *ad libitum*. All the animal experimental protocols were followed in accordance with the Indian National Law on animal care and use and approved by the Institutional Animal Ethics Committee of SASTRA University (CPSEA approval No.: 240/SASTRA/IAEC/RPP).

2.3. DMH and phytochemical dosage and administration

DMH at a dose of 40 mg/kg body weight was dissolved in 1 mM EDTA, pH was adjusted to 6.5 with 1 mM NaOH and was given subcutaneously once a week for 5 weeks starting from the first week of the experimental period. *p*-CA at a dose of 50 mg, 100 mg and 200 mg were suspended in 0.5% carboxymethylcellulose (CMC) and administered everyday via intragastric intubation for the total experimental period of 15 weeks.

2.4. Experimental design

Rats were randomly divided into six groups (n = 7/per group). Group 1 (control group) was injected subcutaneously with normal saline once a week for five weeks and fed orally with 0.5% CMC for 15 weeks. Group 2 served as *p*-CA control (200 mg/kg b.w.) received oral administration of *p*-CA for 15 weeks. Group 3 served as DMH control (40 mg/kg b.w. s.c. once a week for 5 weeks). In addition to DMH, Group 4, Group 5 and Group 6 received oral administration of *p*-CA at a dose of 50 mg, 100 and 200 mg/kg b.w. respectively for 15 weeks. The total body weight was measured and recorded once per week. The experimental design is represented in Fig. 1 After the experimental period of 15 weeks the rats were sacrificed by CO_2 asphyxiation and subjected to necropsy.

2.5. Macroscopic observations

Colon was removed and flushed with ice cold PBS to remove debris. The colons were cut longitudinally and placed on clean tissue paper. The numbers of polyps were documented.

2.6. Determination of aberrant crypts

The colonic tissues were flushed with ice cold PBS to remove debris and excised longitudinally. ACF was performed according to the method described by Bird et al. [10] Briefly the colon was cut in to proximal and distal. The sections were placed in between filter paper in a petri plate containing 10% neutral buffered formalin for 24 h at 4 °C. The formalin fixed colon was then stained with 0.2% methylene blue for 20 min and then viewed under microscope (Model No. Leica ST4040). The same colon was further stained with 0.2% methylene blue for 20 min and then soaked in 70% methanol for destaining and then viewed under microscope (Model No. Leica ST4040) for DACF.

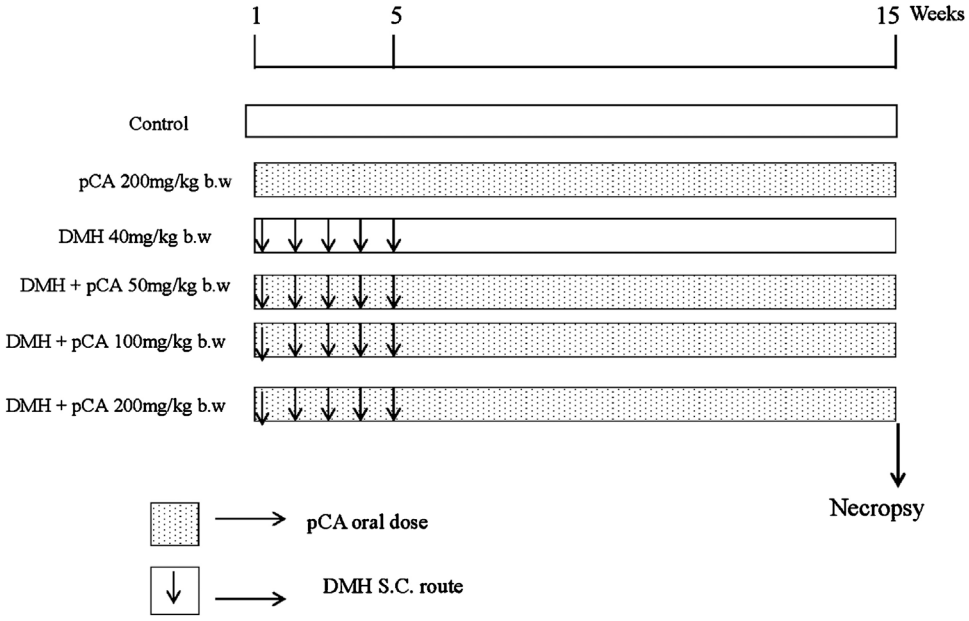

Fig. 1. Schematic representation of Experimental Design.