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Modulatory effect of troxerutin on biotransforming enzymes and preneoplasic lesions induced by 1,2-dimethylhydrazine in rat colon carcinogenesis

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ABSTRACT

Colon cancer is the third most global oncologic problem faced by medical fraternity. Troxerutin, a flavonoid present in tea, coffee, cereal grains, and a variety of fruits and vegetables, exhibits various pharmacological and biological activities. This study was carried out to investigate the effect of troxerutin on xenobiotic metabolizing enzymes, colonic bacterial enzymes and the development of aberrant crypt foci (ACF) during 1,2-dimethylhydrazine (DMH) induced experimental rat colon carcinogenesis. Male albino Wistar rats were randomly divided into six groups. Group 1 served as control. Group 2 received troxerutin (50 mg/kg b.w., p.o. every day) for 16 weeks. Groups 3–6 received subcutaneous injections of DMH (20 mg/kg b.w.) once a week, for the first four weeks. In addition, groups 4–6 received different doses of troxerutin (12.5, 25, 50 mg/kg b.w., p.o. every day respectively) along with DMH injections. Our results reveal that DMH treated rats exhibited elevated activities of phase I enzymes such as cytochrome P450, cytochrome b5, cytochrome P4502E1, NADPH-cytochrome P450 reductase and NADH-cytochrome b5 reductase and reduced activities of phase II enzymes such as glutathione-S-transferase (GST), DT-diaphorase (DTD) and uridine diphospho glucuronyl transferase (UDPGT) in the liver and colonic mucosa of control and experimental rats. Furthermore, the activities of fecal and colonic mucosal bacterial enzymes, such as β-glucronidase, β-glucosidase, β-galactosidase and mucinase were found to be significantly higher in DMH alone treated rats than those of the control rats. On supplementation with troxerutin to DMH treated rats, the alterations in the activities of the biotransforming enzymes, bacterial enzymes and the pathological changes were significantly reversed, the effect being more pronounced when troxerutin was supplemented at the dose of 25 mg/kg b.w. Thus troxerutin could be considered as a good chemopreventive agent against the formation of preneoplastic lesions in a rat model of colon carcinogenesis.

Introduction

Colorectal cancer is a major cause of morbidity and mortality around the world (Gellad and Provenzale, 2010). As of 2008, it is ranked as the third most commonly diagnosed cancer and is the third leading cause of cancer death in both men and women in the Western world (Siegel et al., 2011). The incidence rate of colorectal cancer is expected to increase substantially in economically transitioning countries including most parts of Asia where the overall risk was formerly low. This increase may reflect the adoption of Western lifestyle and behavior (Center et al., 2009). The incidence of new colon cancer patients in India is increasing gradually (Sinha et al., 2003) there are about 3.5 million cases of cancer of which about 35,000 are found to suffer from colorectal cancer (Srikhande et al., 2007). Colon cancer arises due to various environmental factors, including diet, genetic predisposition and epigenetic alterations in the colonic epithelium (Giovannucci, 2002). Accumulating evidence from epidemiological studies reveal an interplay between diet and the prevalence of gastrointestinal tract tumors, especially colorectal cancer, which can be promoted by a diet rich in fat and meat (Aggarwal, 2008). Several studies from our laboratory as well as others have shown that a high fat diet promotes tumorigenesis in the chemically induced experimental model of colon carcinogenesis (Aranganathan et al., 2009; Bansal et al., 1978; Reddy et al., 1977; Sangeetha et al., 2010). It may be related to the increased concentrations of secondary bile acids within the colon lumen, which may enhance cell proliferation in the colonic mucosa, and have been found to be carcinogenic in animal models (Baijal et al., 1998).

1, 2-dimethylhydrazine (DMH) induced rat colon carcinogenesis is one of the widely studied experimental models in several chemoprevention studies. Repetitive treatment with this methylating agent was reported to produce colon tumors in rodents that exhibit many of the

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cell kinetics, molecular characteristics and pathological features associated with the human colorectal cancer (Ma et al., 1996; Shamsuddin and Phillips, 1981). DMH is metabolized to form azoxymethane (AOM), which is further metabolized to methylazoxymethanol (MAM) by cytochrome P4502E1 in the liver (Sohn et al., 1991). MAM gets conjugated in the liver by glucuronic acid and is released into the colon via bile as MAM glucuronide where it is deconjugated by the actions of gut microbial enzymes especially β-glucuronidase to yield MAM (Rowland, 1988). Thus, from a chemical perspective, the intestinal flora tends to reverse the metabolites produced in the liver. The regenerated MAM is further metabolized to produce electrophilic methyldiazonium ion, which inturn generates carbonium ion that is responsible for the methylation of nucleic acids and acts as a trigger for colon carcinogenesis (Fiala, 1977; Rosenberg et al., 2009).

ACF has been widely used as an early biomarker for colon carcinogenesis and is considered to be a surrogate preneoplastic lesion since it is found in the colon of carcinogen treated rodents and humans. ACF are easily identified in formalin fixed methylene blue stained whole-mount colon preparations under a light microscope. Biochemical, genetic and morphological studies have shown that ACF and colon tumors share similar alterations, further criteria supporting the hypothesis that ACF are precursors of colorectal cancer (Bird, 1995; Bird and Good, 2000; Mori et al., 2005; Rudolph et al., 2005). Therefore, it is important to identify chemopreventive compounds which can block the potential biochemical factors responsible for ACF formation in DMH induced colon carcinogenesis.

Drug development from natural products is currently emerging as a highly promising strategy to identify novel anticancer agents. During the past decade, a large number of phytochemicals from the diet and medicinal plants have been evaluated for anticancer activity due to their ability to interfere with multiple pathways controlling growth and apoptosis of cancer cells (Khan et al., 2008). Many epidemiological studies have reported that high consumption of whole grains, fruits and vegetables is associated with a low risk of colorectal cancer. In addition, much of the attention given to flavonoid compounds comes from the results of epidemiological studies that suggest high fruit and vegetable consumption is associated with the decreased risk of several types of cancer, including breast, colon, lung, larynx, pancreas, oral and prostate cancer (Ross and Kasum, 2002).

Troxerutin (Fig. 1A), a trihydroxyethylated derivative of rutin, known as vitamin P4, is a flavonoid present in tea, coffee, cereal grains and a variety of fruits and vegetables. Troxerutin is an effective scavenger of reactive oxygen species (ROS) and may also function indirectly as antioxidant through its effects on enzyme activities (Blasig et al., 1987, 1988; Fan et al., 2009). Troxerutin in recent times have gained great importance by virtue of its numerous pharmacological and biological properties such as antifibrinolytic (Boisseau et al., 1995), antiinflammatory (Fan et al., 2009), anti γ-radiation (Maurya et al., 2005) and antidiabetic (Chung et al., 2005) effects. Chemoprevention aims to halt or reverse the development and progression of cancerous cells through use of non-cytotoxic nutrients and/or pharmacological agents during the period between tumor initiation and malignancy. Modulation of enzymes involved in metabolic activation and excretion of carcinogens is one of the best-investigated mechanisms for chemopreventive agents. The objective of the present study was to investigate the chemopreventive efficacy of troxerutin employing DMH induced colon carcinogenesis in male albino Wistar rats as an experimental model.

Materials and methods

Chemicals

Troxerutin and 1,2-dimethylhydrazine (DMH), were purchased from Sigma Chemicals Co., St. Louis, MO, USA. All other chemicals and solvents utilized were of analytical grade and obtained from Hi-Media Laboratories Ltd., Mumbai, India.

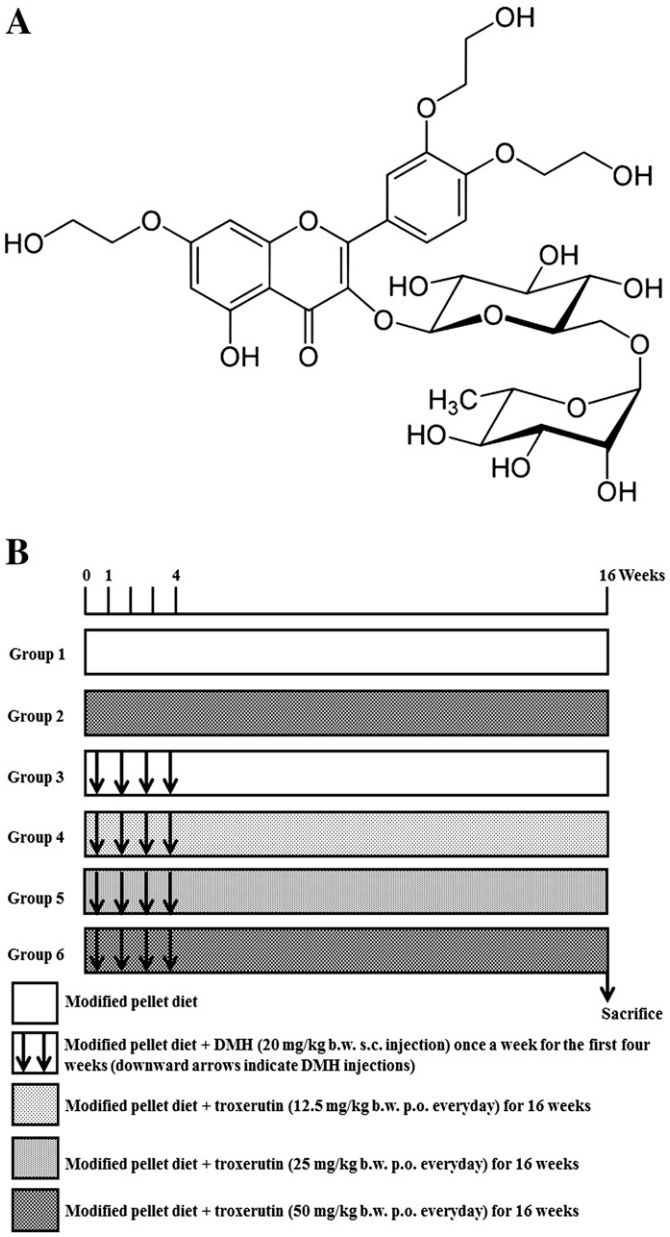

Fig. 1. A. Chemical structure of troxerutin. B. Diagrammatic representation of the experimental design.

Animals and diet

The experiment was carried out using male albino Wistar rats with body weight ranging from 130-150 g (5 weeks old). Thus were procured and maintained at the Central Animal House, Rajah Muthiah Medical College & Hospital, Annamalai University, Tamil Nadu, India. The animals were housed in solid-bottomed polypropylene cages with a wire mesh top and a hygienic bed of husk in a specific-pathogen-free animal room under controlled conditions of a 12-h light/12-h dark cycle, with a temperature of 25 ± 2 °C and relative humidity of 50 ± 10% till the end of the experimental period. Commercial pellet diet containing 4.2% fat (Hindustan Lever Ltd., Mumbai, India) was powdered and mixed with 15.8% peanut oil, making a total of 20% fat in the diet (Table 1). This modified diet and water was fed *ad libitum* throughout the experimental period of 16 weeks. The animals were cared as per the principles and