Experimental and Toxicologic Pathology 64 (2012) 247–251

Contents lists available at ScienceDirect

Experimental and Toxicologic Pathology

journal homepage: www.elsevier.de/etp

Kenaf seed supercritical fluid extract reduces aberrant crypt foci formation in azoxymethane-induced rats

Siti Aisyah Abd Ghafar[a], Latifah Saiful Yazan[b], Paridah Md Tahir[c], Maznah Ismail[a,b,*]

[a] Nutraceutical and Nutrigenomic Programme, Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[b] Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[c] Institute of Tropical Forestry and Forest Products (INTROP), Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

ARTICLE INFO

Article history:
Received 9 March 2010
Accepted 27 August 2010

Keywords:
Hibiscus cannabinus (kenaf)
Kenaf seed
Supercritical fluid extraction
Aberrant crypt foci
Sprague–Dawley

ABSTRACT

Kenaf (*Hibiscus cannabinus*) a plant of the family *Malvaceae*, is a valuable fiber plant native to India and Africa. Kenaf seeds contain alpha-linolenic acid, phytosterol such as β-sitosterol, vitamin E and other antioxidants with chemopreventive properties. In the present study we examined the hypothesis that kenaf seed 'supercritical fluid extract' (SFE) extract could suppress the early colon carcinogenesis in vivo by virtue of its bioactive compounds. To accomplish this goal, 60 male rats were randomly assigned to 5 groups which were (1) negative control group [not induced with azoxymethane (AOM)]; (2) positive control group (induced with AOM but received no treatment); (3) group treated with 500 mg/kg kenaf seed SFE extract; (4) group treated with 1000 mg/kg kenaf seed SFE extract; (5) group treated with 1500 mg/kg kenaf seed SFE extract. At 7 weeks of age, all rats except the negative control group received 15 mg/kg of AOM injection subcutaneously once a week for 2 weeks. Rats were euthanized at 13 weeks of the experiment. Number of ACF (mean ± SD) ranged from 84.4 ± 4.43 to 179.5 ± 12.78 in group 2, 3, 4, 5. ACF reductions compared with the untreated group were 45.3, 51.4 and 53.1% in rats fed with 500, 1000 and 1500 mg/kg body weight, respectively. There were no significant differences in weight gain among groups. Our finding indicates that kenaf seed SFE extract reduced AOM-induced ACF in Sprague–Dawley male rats.

1. Introduction

Colon cancer, also called colorectal cancer or bowel cancer, includes cancerous growths in the colon, rectum and appendix. As of 2008, it ranked as the third most commonly diagnosed cancer and the third leading cause of cancer death in both men and women in the Western world (American Cancer Society, 2010). In Malaysia, data from the 2005 National Cancer Registry indicated that colon cancer is the most common cancer among men whereas among women it is the third (National Cancer Registry, 2005). Colon carcinogenesis is a multistep process involving three distinct stages, initiation that alters the molecular message of a normal cell followed by promotion and progression that ultimately ends up with a phenotypically altered transformed cell (Vinay et al., 2003). Although epidemiological and experimental documentation that high dietary fat intake increases the risk of colon cancer is strong (Dwivedi et al., 2003; Nkondjock et al., 2003) there appears to be evidence that indicate dietary fat, depending on the source, quantity and fat composition is likely to reduce the incidence of colon cancer (Sambanthamurthi et al., 2000).

Aberrant crypt foci (ACF) are putative preneoplastic lesions that appear on the surface of colon rodents and were described as lesions consisting of large, thick crypts in methylene blue stained specimen colon after subsequent treatment with chemically induced colon carcinogens such as azoxymethane (AOM) (Bird, 1995). These preneoplastic lesions, which occur in the colonic mucosa of rodents, have also been observed at a higher frequency in the colons of patients with sporadic and inherited forms of colon cancer (Bird, 1995; Pretlow et al., 1992a,b; Roncucci et al., 1991; Bunpo et al., 2004). ACF are considered putative preneoplastic lesions because they share many morphological and biochemical characteristics of tumors, including a comparable increase in cell proliferation, higher expression of tumor associated antigens and dysplasia (Pretlow et al., 1994a,b; Sim et al., 1997). The molecular features of ACF defining them as colonic preneoplastic lesions have been studied exten-

Abbreviations: ACF, aberrant crypt foci; ACUC, Animal Care and Use Committee; ANOVA, analysis of variance; ALA, α-linolenic acid; AOM, azoxymethane; KSSE, kenaf seed SFE extract; SD, standard deviation; SFE, supercritical fluid extraction.

* Corresponding author at: Laboratory of Molecular Biomedicine, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. Tel.: +60 3 89472115; fax: +60 3 89472116.
E-mail addresses: aisyah0610@yahoo.com, myhome.e@gmail.com (M. Ismail).

0940-2993/$ – see front matter © 2010 Elsevier GmbH. All rights reserved.
doi:10.1016/j.etp.2010.08.016

sively (Cheng and Lai, 2003). Using ACF as a model for short term screening assay for colon carcinogenesis in laboratory rodents has so far proven to be a reliable biomarker (Williams et al., 2007; Ozkal et al., 2005; Boateng et al., 2007; No et al., 2007).

Kenaf (*Hibiscus cannabinus*) a plant of the family Malvaceae, is a valuable fiber plant native to India and Africa (Mohamed et al., 1995). Kenaf is one of the allied fibers of jute and shows similar characteristics. This plant contains various active components including tannins, saponins, polyphenolics, alkaloids, essential oils and steroids, which have long been prescribed in traditional folk medicine in Africa and India (Agbor et al., 2005; Kobaisy et al., 2001). Kenaf seeds yield vegetable oil that is edible for human consumption (Mohamed et al., 1995). Kenaf seed oil (extracted by soxhlet extraction) contains alpha-linolenic acid (ALA), the essential omega-3 fatty acid that is metabolized to eicosapentaenoic acid, a precursor of eicosanoids with anti-inflammatory and antithrombotic activity and also known as chemopreventive agent (Williams et al., 2007; Mohamed et al., 1995; Ruiz et al., 2002). Additionally, kenaf seed oil also contains phytosterol which possesses anti cancer, anti oxidant and lipid lowering cholesterol properties (Kritchevsky and Chen, 2005; Berger et al., 2004; Choi et al., 2003). However, oils that are extracted from organic solvents such as n-hexane or petroleum ether are always doubted for its safe consumption due to the incomplete solvent removal. Hence, supercritical fluid carbon dioxide extraction offers a better way of extraction.

Supercritical fluid extraction is the process of separating one component from another using supercritical fluid as the extracting solvent. A supercritical fluid is any substance at a temperature and pressure above its thermodynamic critical point. It has the unique ability to diffuse through solids like a gas, and dissolve materials like a liquid (Wang and Weller, 2006). Additionally, it can readily change in density upon minor changes in temperature or pressure. Moreover, it also enables the oil extraction to be carried out at low temperature and allows for complete removal of the solvent at the final stage of extraction (Ozkal et al., 2005). Compared with liquid solvents, SFE have several more advantages: (1) the dissolving power of a supercritical fluid solvent depends on its density which is highly adjustable by changing the pressure or/and temperature; (2) supercritical fluid has a higher diffusion coefficient and lower viscosity and surface tension than a liquid solvent, leading to a more favorable mass transfer. These properties make it suitable as a substitute for organic solvents in a process called supercritical fluid extraction (SFE) (Wang and Weller, 2006).

Although kenaf has been widely used traditionally to ease various symptoms and treat diseases, few studies on its chemopreventive activity have been reported. In the present study, we hypothesized that kenaf seed SFE extract may suppress colon carcinogenesis in an animal model. Hence this study was designed to meet two objectives. The first was to extract kenaf seed using supercritical fluid extraction (SFE) and the second was to examine the chemopreventive effect of kenaf seed SFE extract against early chemically induced azoxymethane (AOM) Sprague–Dawley male rats by determining the incidence of aberrant crypt foci (ACF) histologically.

2. Methods and materials

2.1. Preparation of kenaf seed SFE extract

Kenaf seed, V36 was purchased from the Malaysia Kenaf Tobacco Board, Pasir Putih, Kelantan. Kenaf seed was cleaned and dried at constant temperature (50 °C) overnight in oven (FD 115, Fisher Scientific). The final moisture content of the dried seeds was less than 5%. The dried seeds were stored at 4 °C until further use. Extracts of kenaf seed were prepared using the supercritical carbon dioxide fluid extractor (Thar 1000 F, USA) at 600 bars, 40 °C. This was done following a method developed in our lab as described by Chan and Maznah (2009) with slight modifications. In brief, 100 g of seed were ground using a stainless steel blender (Waring Commercial, USA) for 1 min and placed into a 1 l extraction vessel. The desired temperature and pressure were then set. The flow rate of carbon dioxide was set at 25 g/min and regulated by automated back pressure regulator. The extraction started after the desired temperature and pressure were obtained. The whole extraction process lasted for 2.5 h and the yield obtained was calculated.

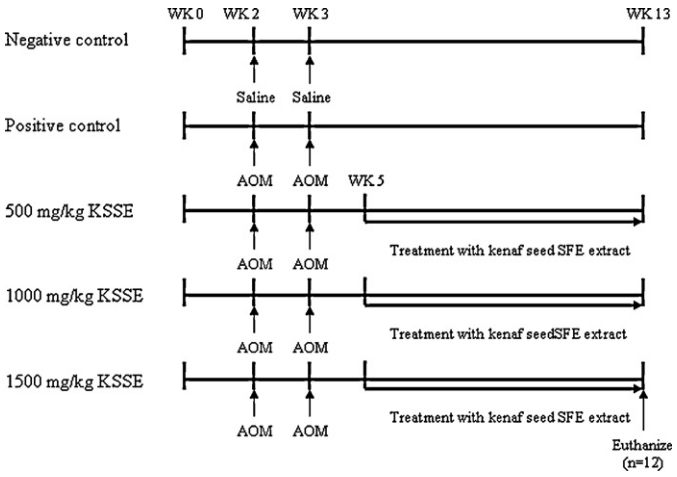

Fig. 1. Experimental design of the study. The rats were randomized into five groups. Group 1 is not treated with AOM, whereas groups 2–5 were injected with 15 mg/kg body weight subcutaneously with AOM at week 2 and 3. The experimental groups were as follow: negative control group (group 1), positive control group (group 2), 500, 1000, 1500 mg/kg fed with kenaf seed SFE extract (KSSE) (groups 3–5).

2.2. Animal management

Ethics approval for animal use in this study was obtained from the Animal Care and Use Committee (ACUC), Faculty of Medicine & Health Sciences, Universiti Putra Malaysia (ACUC no: UPM/FPSK/PADS/BRUUH/00256). Sixty male Sprague–Dawley rats (at 4 weeks of age) weighed between 90 and 150 g were divided randomly into five groups ($n = 12$) after 2 weeks of acclimatization period. They were kept in a well-ventilated room at the animal house under room temperature, 29–32 °C, 70–80% humidity with a 12 h light/dark cycle. The animals were fed with a basal diet for 13 weeks. The animals were cared for according to the guidelines of the Animal Care and Use Committee (ACUC) Faculty of Medicine & Health Sciences Universiti Putra Malaysia. They were divided into groups as follow (refer to Fig. 1): negative control group (group 1), positive control group (group 2), fed with 500, 1000 and 1500 mg/kg kenaf seed SFE extract (groups 3–5), respectively. Groups 3–5 were treated with kenaf seed SFE extract via gavage daily in the morning for 13 weeks. Water and food were given *ad libitum*. Body weight was recorded weekly.

2.3. Administration of carcinogen

At 7 weeks of age, rats received the first azoxymethane (AOM) injection (Sigma) followed by the second injection in the following week. This subcutaneous administration of AOM was given at a dosage of 15 mg/kg to all treatment groups with the exception of negative control (as shown in Fig. 1).