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Attenuation of histopathological alterations of colon, liver and lung by dietary fibre of barley Rihane in azoxymethane-treated rats

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ABSTRACT

This study was carried out to determine the effect of dietary fibre (DF) of barley Rihane (BR) in the attenuation of colon, liver and lung histopathology alterations induced by azoxymethane (AOM) in rats. Rats were fed a control (C) or experimental diet containing 30% of BR. The intended rats for cancer treatment received two successive subcutaneous injections of azoxymethane (AOM) at 20 mg/kg body weight. The colons were analyzed for crypt multiplicity after 12 weeks of treatment. A histological study of the colon, liver and lungs was determined. The results showed that the BR diet significantly reduced the number of aberrant crypt per focus and altered their distribution. In addition, DF of BR increased significantly the mucus secretion compared to control group. The use of the AOM as colon specific carcinogen substance altered the liver and lung architectures, whereas the presence of DF of BR could be a protective factor for these organs.

1. Introduction

Colon cancer is a malignant tumor, and is recognized as the third most common cancer worldwide, with high morbidity and mortality (Haggar & Boushey, 2009). Many epidemiological and experimental studies have suggested an important role for dietary fibre (DF) of cereals in the prevention of colon cancer (Martinez, Marshall, & Giovannucci, 2008; West, Boustiere, Fischbach, Parente, & Leicester, 2009). Barley (*Hordeum vulgare* L.) is an interesting source of dietary fibre. It is an ancient cereal grain, which upon domestication has evolved from largely a food grain to a feed and malting grain (Oscarsson, Andersson, Salomonsson, & Åman, 1996). However, barley food use today remains important in some cultures around the world, particularly in Tunisia which is the second centre of diversity for barley. In Tunisia, barley is used for both feed (85%) and food (15%), with the local variety preferred for food preparations rather than the improved cultivars (El Felah & Medimagh, 2005). Rihane, a Tunisian barley variety, officially registered in 1987, has contributed significantly to the increase in the national production of barley in Tunisia, and it is cultivated in Morocco, Algeria, Libya, Lebanon, Iraq, Iran, Afghanistan, Cyprus and China (Abdellaoui, Kadri, Ben Naceur, & Bettaib Ben Kaab, 2010). Azoxymethane (AOM), an active metabolite of 1,2-dimethylhydrazine (DMH) is a colon-specific carcinogen, which serves as an effective tool to assess colon tumors in susceptible rodents. The most affected organs by this carcinogen are the liver and lungs during the induction of colon cancer (Chan, Cook, & Stanners, 2006). Aberrant Crypt Foci (ACF) are early pre-neoplastic lesions of adenocarcinoma that appear on the surface of rodents after subsequent treatment with chemically induced colon carcinogenesis, such as azoxymethane (AOM) (Bird, 1995; Caderni, Femia, & Giannini, 2003). ACF appear as a single focus, that are characterized by more than one crypt, they possess thickened epithelia with altered luminal openings and are more elevated than normal crypts when there are viewed under a microscope (Bird, 1987).

Even though barley is widely known due to its nutritional potential, in Tunisia, there is no published data about the effect of Tunisian barley variety on health especially on colon cancer. Considering the large amounts of dietary fibre (DF) of barley Rihane (BR), the objective of this study was to investigate the beneficial effects of these DFs on aberrant crypt formation in the rat colon carcinogenesis induced by AOM. In addition, the effects on the

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attenuation of colon, liver and lung histopathology alterations were also evaluated.

2. Materials and methods

2.1. Barley Rihane

The "Rihane" variety, six-rowed barley, was provided by the Field Crop Laboratory of INRAT. This variety was grown in 1.5/50 m head-rows plots at the experimental station of Beja's agriculture, 100 km North-West of Tunisia.

2.2. Animals, housing and diets

Adult male Wistar rats were purchased from the Central Pharmacy (Pharmaceutical Industries Society, Tunis-Tunisia). The rats were housed two per cage in a temperature-controlled room (22–24 °C) with a 12 h light–dark cycle (lights on 8:00–20:00) and humidity rate (70 ± 4%). They were allocated randomly into four groups (n = 10 per group) with approximately equal body weights and given free access to water and diet throughout the study. The diet composition is shown in Table 1. The diet was prepared as pellets (Provital, Grombalia, Tunisia). The control diet was a modified form of the AIN-76 diet (AIN, 1977). An experimental diet containing 30% of the BR was prepared by a partial substitution of corn and soybean meal (used in the control diet). Diets were isonitrogenous and isoenergetic. However DF content was almost doubled in the experimental diet (total dietary fibre was 8.69% in control diet and 15.2% in BR diet).

2.3. Carcinogen

Azoxymethane (AOM), a colon carcinogen, was purchased from Sigma Chemical Co. AOM was dissolved in saline just before injection.

2.4. Experimental design

After two weeks of adaptation, animals were divided into four groups. The rats were fed the experimental diet for a week, and then received 2 injections of AOM (Sigma Chemicals, St Louis, MO) at 7 and 8 weeks of age, according to the standard protocol established by Martha, Lloyd, Louis, and Chawan Chandramohan (2005). The carcinogen was administered subcutaneously in saline at 20 mg/kg body weight. The negative controls were injected with saline. Body weights (wt) and food intake were measured twice weekly. Twelve weeks after the first injection ten animals from each group were killed, their colons removed and examined for aberrant crypts formation as outlined below.

All experiments were carried out in compliance with the rules of the Tunisian Society for the Care and Use of Laboratory Animals.

Table 1
Composition of experimental diets (% DM).

	C	BR
Diet ingredients (%)		
Corn starch	68	40
Soya bean meal	27	25
Barley	–	30
Mineral mix[a]	3.5	3.5
Vitamin mix[a]	1.0	1.0
DL-Methionine	0.5	0.5
Chemical composition (% *DM*)		
Crude protein	17.64	17.58
Total dietary fiber	8.69	15.24
Total energy (kcal/kg DM)	2867	2800

C: Control diet. BR: barley Rihane diet.
[a] AIN-76 vitamin and mineral mixtures (AIN, 1977).

All experiments were conducted at the animal facilities at the Faculty of Medicine, Monastir, Tunisia; with the approval of the Faculty of Medicine Ethics committee.

2.5. Crypt multiplicity (aberrant crypts/focus) analysis and colon histological

Analysis of aberrant crypts formation was performed as previously described by Norazalina, Norhaizan, Hairuszah, et al. (2010). Briefly, colons were fixed in 10% buffered formalin, stained with 0.2% methylene blue, and aberrant crypt foci were scored under a light microscope at 40× or 100× magnifications. Aberrant crypts were distinguished from normal crypts by their increased size, prominent epithelial cells and increased pericryptal area according to established criteria (Norazalina, Norhaizan, Hairuszah, et al., 2010). The numbers of aberrant crypts (ACs) per focus were quantified. Large aberrant crypt foci were defined as containing 4 crypts or more. After the aberrant crypts formation analysis, the colonic mucosa was embedded in paraffin and serially cut perpendicular to the surface for histological examination with Hematoxylin and Eosin (H&E) staining and Alcian Blue (AB)/Periodic Acid Shiff (PAS) stain (Mucus secretion detection).

2.6. Organ collection for histologic studies

The lungs and liver were collected for histologic studies. The right lung from each rat was inflated with 10% buffered formalin and then placed in the same solution and fixed for 1 week. Sagittal sections were embedded in paraffin, and 4-μm thick sections were stained by Hematoxylin and Eosin for light microscopy.

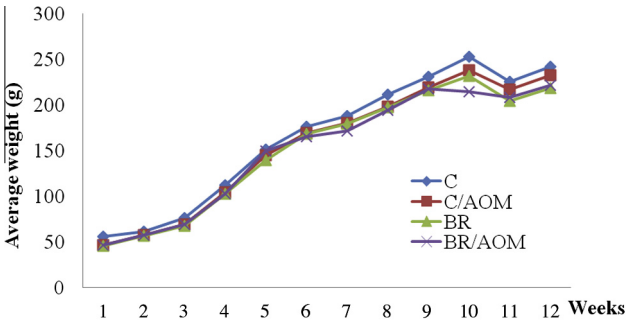

Fig. 1. Changes in body weight of experimental rats. C: rats fed the control diet. C/AOM: rats fed the control diet and treated with azoxymethane. BR: rats fed the barley Rihane diet. BR/AOM: rats fed the barley Rihane diet and treated with azoxymethane.

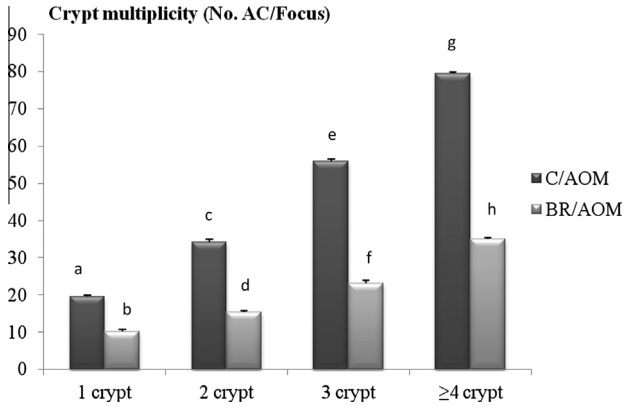

Fig. 2. Effect of basal diet containing 30% BR on crypt multiplicity in azoxymethane-treated rats compared to control[a]. Each value is the mean of ten rats ± SEM. Figures having different lowercase letters (a, b, c, d, e, f, g, h) are significantly different ($p < 0.05$). BR: rats fed the barley Rihane diet. BR/AOM: rats fed the barley Rihane diet and treated with azoxymethane.