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Propolis aqueous extract preserves functional integrity of murine intestinal mucosa after exposure to ionizing radiation

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ABSTRACT

The ability of a specially prepared water propolis extract (PWE) to preserve the functional activity of the intestinal mucosa after radiation exposure was studied. PWE was given orally (650 mg/kg) to rats five days prior to irradiation by 6 Gy and continued for further two days. Rats were sacrificed 24 h later, intestinal segments were examined histologically and homogenates were used to assess relevant biochemical parameters reflecting intestinal injury. Irradiation led to a rise in the histological damage score, a rise in tissue TNF-α and TBARS, and a decrease in sucrase, alkaline phosphatase, GSH and cholecystokinin as well as a decrease in plasma citrulline. The findings reflect a decrease in intestinal functional activity. PWE preserved the intestinal integrity and largely protected against the changes induced in the histology damage score and all parameters measured, possibly as a result of the antioxidant and anti-inflammatory action of its caffeic acid content.

1. Introduction

Ionizing radiation (IR) is often used as a therapeutic measure to treat certain malignancies, but treatment is often limited by damage to normal tissues, particularly those of a rapidly proliferating nature such as the intestinal mucosa (Hamama et al., 2014). Intestinal injury is usually initiated by the production of reactive oxygen species (ROS) which then induces damage to the mucosal and submucosal tissues (Ong et al., 2010) and to villus atrophy and loss of their functional integrity (Du et al., 1994). Criteria for assessing the functional integrity and absorptive capacity of the small intestine include measuring the tissue level of sucrase, intestinal alkaline phosphatase (IAP) enzymes and cholecystokinin (CCK). Sucrase and IAP are normally secreted by the brush border membrane and have been shown to be affected by intestinal damage (Petschow et al., 1993; Naruhashi et al., 2000; Tooley et al., 2006) while CCK is secreted from the endocrine glands of the GI tract (Hauer-Jensen et al., 2007) and was found to be reduced by radiation exposure, thereby affecting gastric emptying and bowel motility (Rozengurt et al., 2002). The decrease of intestinal absorptive function following irradiation has also been correlated to the loss of functionally active enterocytes lining the absorptive mucosal surface leading to a decrease in plasma level of citrulline (Overgaard and Matsui, 1990; Lutgens et al., 2003).

Many natural products have been studied in an attempt to find agents that can preserve the integrity of the intestinal mucosa in the face of radiation exposure (Lalla et al., 2008). In this study, attention was focussed on the potential usefulness of propolis, a resinous substances produced by honey bees, that has been shown to have a wide variety of biological properties, including antibacterial (Bankova, 2005) and antioxidant (Heim et al., 2002) activities. Ethanolic and aqueous extracts of propolis have been reported to protect against radiation-induced mucositis of the oral cavity (Ghassemi et al., 2010; Benderli and Deniz, 2011; Motallebnejad et al., 2014), but to the best of our knowledge the effect of propolis extracts on the integrity and functional activity of the small intestine has not been previously reported. The activity of these extracts has been attributed mainly to their content of flavonoids and caffeic acid derivatives. The present study, however, deals with a specially prepared water extract of propolis (PWE) containing mainly caffeic acid and only traces of flavonoids in preserving the integrity and functional activity of the small intestine in the face of exposure to radiation. Histological examinations as well as measurement of relevant parameters indicative of intestinal functional integrity have been studied.

2. Materials and methods

2.1. Animals

Male Wistar rats, each weighing 120–150 g, were obtained from the animal breeding unit of the National Research Centre (Dokki,

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Giza, Egypt). Rats were left to acclimatize in the animal facility of the National Centre for Radiation Research and Technology (NCRRT) – Atomic Energy Authority (Nasr City, Cairo, Egypt) for at least one week prior to experimentation. They were provided with the standard pellet diet, given water ad libitum, kept at a temperature of 22 ± 3 °C, 60–70% relative humidity and a 12 dark/light cycle throughout the experimental period. The study was conducted in accordance with the guidelines set by the European Economic Community (EEC) regulations (Revised Directive 86/609/EEC) and approved by the Ethical Committee for Animal Experimentation at the Faculty of Pharmacy, Cairo University with PT (84).

2.2. Irradiation of animals

Whole body irradiation of rats was carried out at the NCRRT using a Gamma cell-40 biological irradiator furnished with a Cesium^{137} source (Atomic Energy of Canada Limited, Mississauga, Ontario, Canada). Irradiation was carried out on non-anaesthetized rats placed in the plastic sample tray of the Gamma cell-40 and exposed to a radiation dose level of 6 Gy, delivered at a rate of 0.46 Gy/min. The dose of irradiation was chosen after carrying out preliminary experiments with lower and higher doses of irradiation. Six Gy was chosen as suitable for inducing adequate intestinal mucositis for the purpose of this study (results not shown).

2.3. Drugs and chemicals

PWE was generously provided by Propolis Research Centre A/S (Blistrup, Denmark) as a lyophilized powder prepared to contain not less than 0.05% organic aromatic acids (caffeic acid, ferulic acid and cinnamic acid) and only traces of flavonoids. A voucher specimen of the extract has been deposited in the Pharmacology Department, Faculty of Pharmacy, Cairo University, and preserved in a refrigerator for future reference. A chromatogram of the extract was supplied by the manufacturers showing that the main constituents were caffeic acid, vanillin, ferulic acid, isoferulic acid, cinnamic acid and met-cinnamic acid (Fig. 1). Inter-batch differences in composition have also been shown by the manufacturers to be minimal. The extract was dissolved in distilled water in a concentration/volume suitable for oral administration to rats.

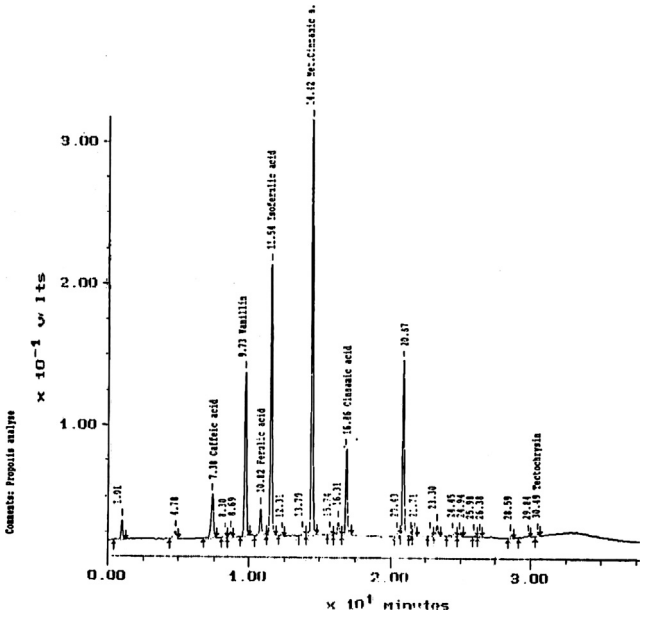

Fig. 1. HPLC for propolis water extract (PWE) showing main constituents of the extract.

All fine chemicals and reagents were purchased from Sigma–Aldrich (St Louis, MO, USA) or were of the purest analytical grade available. Enzyme-linked immunosorbent assay (ELISA) kits specific for rats for citrulline were purchased from Cusabio Biotec Co., Ltd. (Wuhan Hubei, China), for TNF-α from ID Labs Biotechnology (London, Ontario, Canada) and for CCK from Wuhan ElAab Science Co. Ltd. (Guangguguoji, China)

2.4. Experimental design

The rats were blindly allocated to three groups, each consisting of eight rats. One group was exposed to irradiation but left untreated, one group was treated orally with PWE in a dose of 650 mg/kg for 5 days before irradiation and continued for 2 days after, while the third group served as normal control animals (untreated and not exposed to radiation).

Animals were sacrificed on the third day after irradiation. The dose of the extract was chosen on the basis that it is equivalent to a dose of 5 ml/kg of a 13% aqueous propolis extract reported in the literature to have anti-inflammatory and antioxidant effects (El-Ghazaly and Khayyal, 1995; El-Ghazaly et al., 2011). This was further confirmed by a pilot study carried out using three different doses of PWE (450 mg/kg, 650 mg/kg and 850 mg/kg) to study their effect on the histological alterations induced in the jejunum by the chosen radiation dose (overall damage severity "ODS" score... see Section 2.5). PWE induced a dose-dependent protective effect (Fig. 3). The dose of 650 mg/kg was selected as being adequate: the intestinal mucosa and the villi appeared nearly normal after treatment (Fig. 2D).

Blood was collected for the separation of plasma, which was then stored at −20 °C until required. Segments of jejunum were dissected out and fixed in 10% formalin for histological examination. Another part of the jejunum tissue was flushed of its contents, washed with ice-cold isotonic saline and used to prepare 10% homogenates in different media according to the parameters to be measured using a Glas Col® homogenizer (Terre Haute, IN, USA). The parameters for functional integrity included plasma citrulline, intestinal sucrase and alkaline phosphatase as well as intestinal CCK. In addition, oxidative stress markers, TBARS and reduced GSH, and an inflammation marker, TNF-α, were measured in intestinal homogenates.

2.5. Histological examination

The jejunal segment fixed in formalin was embedded in paraffin wax, sectioned serially into 4 μm thick sections, and stained with hematoxylin–eosin (HE). The specimens were examined by a pathologist blinded to the treatment protocols under a Leica Aristoplan microscope (Leica, Bensheim, Germany) and images were captured with a charge-coupled device camera (Visitron Systems, Puchheim, Germany) at 100× and 400× magnifications. A semi-quantitative histological assessment of intestinal damage was carried out to obtain an overall damage severity (ODS) score (Howarth et al., 1996). Briefly, a total median score of 9 criteria was computed for each intestinal section. Each criterion was graded from 0 (normal) to 3 (severe damage), so that the maximal damage score that could be reached for any section was 27. These criteria comprised villus fusion and stunting (atrophy), reduction in goblet cell number, epithelial denudation and erosion, activation of glandular epithelium, activation of nuclei of enterocytes, inflammatory cell infiltration, edema and hemorrhage in lamina propria, and the number of apoptotic bodies.