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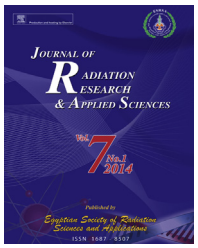

Microbial regulation and protective effects of yerba mate (*Ilex paraguariensis*) in gamma-irradiated mice intestine

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ABSTRACT

The small intestinal epithelium is highly sensitive to radiation and a major site of injury during radiation therapy and environmental overexposure. The objective of this study was to evaluate yerba mate extract (MT) as a potential radioprotective agent in the intestine. First yerba mate was exposed to doses of gamma radiation and the extract was analyzed for its antimicrobial, antioxidant and total phenolic compounds. Male mice were divided into 4 groups: Control group: received saline, MT group: received MT, Irradiation group: exposed to 8 Gy of gamma radiation, MT+ irradiation group: pre-treated with MT then exposed to gamma radiation. The results obtained *in vitro* showed that, MT of gamma irradiated yerba mate showed a reduction in antioxidant activity and total phenolic compounds compared to control. MT exerted antimicrobial activity with minimum inhibitory concentration (0.5 mg/ml) for *Lactobacillus*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis*. *In vivo*, mice exposed to gamma radiation, showed significant reduction in the total microbial count and disappearance of *E. coli*, induction of lipid peroxidation, protein carbonyl, advanced oxidation protein products, and a decrease in glutathione, also decrease in the activity of lysozyme, catalase and superoxide dismutase, induction in cytokine levels of TNF-α and IL-6 with DNA fragmentation in the intestine, while irradiated mice pre-treated with MT showed, normalized lactic acid bacteria count and diminished pathogenic bacteria as *S. aureus* and *E. coli* also showed ameliorative effect of MT on lipid peroxidation, glutathione, protein carbonyl, advanced oxidation protein products, also normalized the activity of lysozyme, catalase and superoxide dismutase, and ameliorated cytokine levels of TNF-α and IL-6 compared to control, with radioprotection effect on the DNA of intestinal tissue. Our data suggested that, pre-treatment with MT may protect the small intestine from gamma radiation damaging effects showed in the measured biochemical parameters also maintained the microbial flora of lactic acid bacteria and removed pathogenic bacteria and may replace antibiotics description for radiotherapy patients since it showed antimicrobial effect against pathogenic bacteria.

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1. Introduction

Accidental total body exposure to ionizing radiation or radiotherapy used for cancer treatment induce serious damage to normal tissues and may restrict the therapeutic doses of radiation and thereby limits the effectiveness of the treatment. Small intestine epithelium and bone marrow are highly sensitive to radiation and are the major sites of injury during radiation therapy. Diarrhea induced by radiation of small intestine is the limiting factor in the dosing of radiation therapy for rectal cancer and other abdominal malignancies. There is a need for agents that could be given before radiation therapy that would diminish radiation injury to the small intestine without decreasing the radiation sensitivity of the tumor (Baliga et al., 2012; Williams et al., 2010).

Radiotherapy for malignant human neoplasms is a relatively safe and effective form of treatment, but it may become limited by its undesired side effects upon the gastrointestinal tract. The undesired effect of radiation upon normal intestinal tissue as reduction in host defense mechanisms, and changes in the intestinal flora may lead to bloodstream invasion by the microorganisms normally resident in the intestinal tract. Bacteria translocation principally from the intestinal lumen but also from other mucous membranes plays a major role in radiation death. These organisms can be recovered from the blood and organs of animals dying after exposure to lethal doses of total-body irradiation and by the reduction of death rates by treatment with antibiotics. In fact anti-infection therapy is beneficial for human. However, it was reported that gut flora is required for intestinal homeostasis regulation; also bacteria depletion after using broad spectrum antibiotics treatment was found to induce adverse effects (Elliott & Ledney, 2012).

The intestinal microflora is a complex ecosystem containing over 400 bacterial species. Anaerobes outnumber facultative anaerobes. The flora is sparse in the stomach and upper intestine, but luxuriant in the lower bowel. Bacteria occur both in the lumen and attached to the mucosa, but do not normally penetrate the bowel wall. A human body is inhabited by huge numbers of various microorganisms. The largest colonies of microbes live in our digestive system. Some of the gut flora's important functions include: Fermentation of non-digestible carbohydrates, producing short-chain fatty acids, vitamin synthesis, development and control of the immune system, protection against pathogens (Southwick, 2008).

Within the last two decades, a surge in research examining this botanical for its use in human health has occurred, with a more recent focus on its antimicrobial activity. It has been researched mainly for its antioxidant, anticancer, anti-inflammation, and anti-atherosclerosis qualities (Anesini, Ferraro, & Filip, 2006; Heck & de-Mejia, 2007). However, little research has been conducted to determine the compounds responsible for antimicrobial activity from yerba mate (Burris, Davidson, Stewart, & Harte, 2012).

2. Materials and methods

2.1. Plant material and gamma irradiation

Yerba mate obtained from local retailer market, undamaged and disease-free berries was snippet from clusters. Yerba mate were packaged in tightly sealed polyethylene bags, (each bag weighing Ca.150 g) and stored in room temperature till irradiation. For irradiation, yerba mate samples were exposed to gamma irradiation at dose levels of 1, 3 and 5 kGy using ^{60}Co from unit Gamma Chamber 4000, at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Egypt. The dose rate at the time of experimentation was 2.5 kGy/hr.

2.2. Preparation of MT

Yerba mate extract was obtained by macerated 25 g of dried yerba mate in 100 ml absolute methanol in darkness for 24 h at room temperature then filtrated. Methanolic extract filtrate was dried at 40 °C using a rotary evaporator (Buchi R 110, Frawil, Switzerland) (Maha & Siree, 2009). The dry extract was re-dissolved in distilled water and used for microbiological and biological studies.

2.3. Total phenolic content

The total phenolic compounds present in the non-irradiated and irradiated MT were determined by Folin–Ciocalteu colorimetric method (Yasoubi, Barzegar, Sahari, & Azizi, 2007), using spectrophotometer (Spectronic Genesys-5, Thermo Electron, USA).

2.4. Antioxidant activity study (DPPH free radical-scavenging assay)

The scavenging effect on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined by the modified method of Wang, Zhang, and Yang (2005).

2.5. Bacterial cultures

Pathogenic microorganisms used were of Gram positive bacteria: *Bacillus megatrium* (*B. megatrium*), *Bacillus subtilis* (*B. subtilis*), *Staphylococcus aureus methicillin resistant*, *Staphylococcus aureus* (*S. aureus*), *Streptococcus faecalis* (*St. faecalis*) and *Lactobacillus* sp. and Gram-negative bacteria: *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). Microbial isolates were clinical isolates isolated from Ain Shams Specialized Hospital, Cairo, Egypt. The bacterial cultures were maintained on Tryptone Glucose Yeast extract agar (TGY, Difco, USA) slants at 4 °C with a subculture period of 15 days.

2.6. Disc diffusion assay

Antimicrobial activity of MT at (1, 3 and 5 kGy) was investigated against eight bacterial strains by agar disc diffusion method. The previously prepared inoculums were adjusted to 0.5 McFarland standards, which are equal to 1×10^8 CFU/ml and then 0.1 ml was transferred to Mueller Hinton agar (MHA) plates and spread with cotton swabs. Ten microliters of each spice extract were spotted on 6 mm sterile paper discs, leaves for 1 hour until dryness. Ampicillin and 1% dimethyl sulfoxide (DMSO) was used as positive and negative controls, respectively. The plates were incubated overnight at respective