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Effect of radiation hygienization of honey on its health protective properties

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ABSTRACT

Effect of radiation hygienization of honey on its health protective properties in terms of antimutagenicity, anti-cancer and DNA protective properties is reported in this study. Similar to non-irradiated honey, irradiated one too reduced ethyl methanesulfonate induced mutagenicity in *Escherichia coli* cells by ~80% when analyzed by rifampicin resistance assay, but did not induce mutagenesis on its own. Honey displayed strong anti-proliferative property against different cancer cell lines (myeloid leukemia, breast and lung cancer) but did not affect normal cell line (Int-407, intestinal epithelial cell) indicating its differential and selective cytotoxicity. The efficacy of protection against radiation induced oxidative damage was evaluated for plasmid DNA and also bacterial cells. Honey displayed protection to supercoiled form of plasmid DNA at comparatively lower dose of 0.15 kGy and reduced the extent of strand breaks at a higher dose of 1 kGy. Besides, honey also protected the functionality of plasmid DNA which was scored in terms of its transformation efficiency. Honey too protected *E. coli* cells from the radiation induced oxidative damage and D_{10} in the presence of honey increased by ~5 fold (0.1 to 0.49 kGy). Current findings thus indicate the excellent health protective properties of honey and its potential usefulness as a dietary supplement, and also endorse radiation treatment to ensure its safety while maintaining its nutraceutical value.

1. Introduction

Suitability of honey for therapeutic uses always remained under scrutiny due to the presence of microbial spores and other contaminants associated health risks (Snowdon & Cliver, 1996; Nevas et al., 2002; Tosi, Ciappini, Re, & Lucero, 2002). Microbial spores may produce enterotoxins responsible for various diseases including gastro-intestinal disorders (Kotiranta, Lounatmaa, & Haapasalo, 2000; Carlin, 2011; Thwaites, 2014). Hence, consumption of unhygienized honey is not recommended for infants below 1 year and adults with weak immunity (Saxena, Gautam, & Sharma, 2010a; Tanzi & Gabay, 2002). In our earlier findings gamma irradiation (15 kGy) resulted in complete microbial decontamination of honey while retaining its physical and biochemical properties (Saxena et al., 2010a). In another study too medical grade honey was sterilized by radiation treatment (Postmes, van den Bogaard, & Hazen, 1995). Impact of gamma irradiation on

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various qualities of honey such as rheology, color, antibacterial and antioxidant activities have been reported elsewhere (Molan & Allen, 1996; Matsuda & Sabato, 2004; Saxena et al., 2010a). High-temperature treatment of honey for microbial decontamination is generally not accepted due to the possible loss of thermolabile bioactive components such as naturally occurring enzymes like diastase, and increase in the hydroxymethylfurfural (HMF) content (Tosi, Martinet, Ortega, Lucero, & Ré, 2008; Kowalski, 2013). HMF is a cyclic aldehyde formed due to acid catalyzed conversion of hexoses to furans during Maillard reaction or caramelisation process (Capuano & Fogliano, 2011). Heating may also lead to over darkening of honey which is generally not liked by the consumers (Gonzales, Burin, & del Pilar Buer, 1999).

Oxidative stress and environmental mutagens are the root causes of various lifestyle and chronic diseases. Oxidative stress is basically the outcome of imbalance between ROS generation and antioxidants in the organism. In certain cases oxidants can also induce DNA mutations leading to neoplasmic inductions (Wallace, 2002; Thun, DeLancey, Center, Jemal, & Ward, 2010). Dietary antioxidants from health protective functional foods like honey have ability to inhibit some of these deleterious effects of free radicals (Seifried, Anderson, Fisher & Milner, 2007; Kryston, Georgiev, Pissis, & Georgakila, 2011; Forman, Davies & Ursini, 2014). The major components of honey include carbohydrates, proteins, amino acids, enzymes, phenolic compounds including flavonoids, organic acids, and volatile substances, which contribute to its diverse functional properties such as antimicrobial, antioxidant, antimutagenic, antitumor, and anti-inflammatory activities (Bogdanov, Jurendic, Sieber, & Gallmann, 2008).

The objectives of the current study were to assess the health protective properties of honey in terms of antimutagenicity, and antioxidant capacity including *in vitro* radioprotective activities, and to evaluate the effect of radiation treatment on these qualities. The antimutagenic potential was evaluated in terms of its efficacy to inhibit induced mutagenesis caused by ethyl methanesulfonate (EMS) in *Escherichia coli* cells using *rpoB* (RNA polymerase B) based rifampicin resistance assay. The anti-cancer property was tested by assaying anti-proliferative property in different cancer cell lines (myeloid leukemia, breast and lung cancer). The *in vitro* radioprotective property was assayed for its potential to protect a model DNA system (plasmid DNA) as well as bacterial cells from gamma radiation induced oxidative damage.

2. Material and methods

2.1. Honey

Honey samples of major Indian commercial brands (7 in numbers) were used in this study (Saxena, Gautam, & Sharma, 2010b). Most of these samples are blended product collected from local sources from different regions of the country. Fresh honey weighing 250 g, packed, and sealed in glass bottles, were procured and stored (maximum up to 6 months) at 4 °C for analysis. The samples were kept overnight at ambient temperature (26±2 °C) for before analysis.

2.2. Bacterial strain

Escherichia coli (*E. coli*) wild type strain MG1655 (F^- λ^- *ilvG- rfb-50 rph-1*) was used for cell protection experiments as well as antimutagenicity assays, whereas, genetically modified *E. coli* strain DH5α [F^- *endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17*(r_K^- m_K^+), *λ–*] was used for transformation experiments.

2.3. Radiation treatment of honey

It was carried out in a cobalt-60 based Gamma Chamber-5000 (source strength Co^{60}: 260 Tbq, dose rate 6.5 kGy/h) at Bhabha Atomic Research Centre, Mumbai, India. Ceric-cerous sulfate dosimeters were used to measure absorbed dose. The dose uniformity ratio was 1.03. Honey samples (50 ml) were packed and sealed in high-density polyethylene packets, and gamma irradiated at 15 kGy at ambient temperature.

2.4. Antimutagenic potential of honey

An aliquot (1%) of overnight grown *E. coli* MG1655 culture was inoculated in LB broth (25 ml) and grown at 37 °C at 125 rpm up to mid-log phase (~3 h). After that it was incubated on ice for 15 min and centrifuged (8000*g* for 10 min). The pellet was washed with LB broth (50 ml) and resuspended in 25 ml of the same. The cell suspension (5 ml) was incubated with EMS (133 mM) in the absence (control) as well as presence of aqueous solution of honey (250 mg/ml) at 37 °C in a rotary shaker (75 rpm). The cells were then centrifuged and the pellet was washed twice with LB (2 ml) and resuspended in 5 ml of the same. An aliquot (50 μl) of this was inoculated in LB broth (1 ml) in different replicates and incubated overnight on a rotary shaker (125 rpm) at 37 °C. The dilutions of the cultures were spread plated on LB-agar-rifampicin (100 μg/ml) plates for scoring Rif^R (rifampicin resistant) mutants, and on LB agar plates for enumerating viable cells, and the plates were incubated at 37 °C for 24 h. Similar analysis was also performed with irradiated honey alone where EMS was not added. Mutation frequency was calculated as the ratio of total number of Rif^R mutants per ml to the total number of viable cells per ml. Besides, the frequency of spontaneous mutation was also determined.

2.5. Extraction of honey phenolics through sep-pak column

Phenolics from 5 g of the most potent honey brand (III) were extracted with 25 ml of acidified milliQ water (pH 2.0) by stirring the mixture at ambient temperature for 10 min. This was later passed through a solid phase C18 Sep-Pak cartridge, prior conditioned sequentially with 5 ml of methanol, and 10 ml of milliQ water. Sugars and other polar constituents were washed from the column with 20 ml of water. The column was again washed with 10 ml of water/methanol (87:13, v/v), and later phenolics were eluted with methanol (4 ml). The elute was dried in a vacuum dryer, dissolved in milliQ water (4 ml) and evaluated for anticancer activities as discussed below.